CANCER RESEARCH

VOLUME 7
NUMBER 2
FEBRUARY, 1947

A MONTHLY JOURNAL
OF ARTICLES AND ABSTRACTS
REPORTING CANCER RESEARCH

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THE OFFICIAL ORGAN OF THE
AMERICAN ASSOCIATION FOR CANCER RESEARCH, INC.

FOURTH INTERNATIONAL CANCER RESEARCH CONGRESS,
St. Louis, Missouri, September 2-7, 1947.

CANCER RESEARCH

This journal is sponsored by the American Association for Cancer Research, Inc., The Anna Fuller Fund, The Donner Foundation, Incorporated, Cancer Research Division, and The Jane Coffin Childs Memorial Fund for Medical Research.

Published by The Donner Foundation, Incorporated, Cancer Research Division.
Publication Office, 125 Temple Street, New Haven 10, Connecticut

The annual subscription rates for one volume are: To members of the American Association for Cancer Research, Inc., $5.00; to others and to libraries, institutions, and organizations, $7.00. Business communications, remittances, and subscriptions should be addressed to Theodore P. Eberhard, Business Manager, 2136 Land Title Building, Philadelphia 10, Pa.

Entered as second class matter December 16, 1946, at the Post Office at New Haven, Conn., under the Act of March 3, 1879.

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Sex Hormone Effects on Chromosome Size in Leukemic and Normal Lymphocytes of C58 Mice

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(Received for publication September 14, 1946)

In a study of chromosomal enlargement in neoplasms, it had been observed that metaphasic chromosomes of the cells of 6 long-transferred lines of lymphatic leukemia were usually larger in male host mice than in female (12). This sexual difference suggested an investigation of the effect of sex hormones on chromosome size in leukemic and normal lymphocytes.

Experiments along several possible lines of attack on the problem were carried out in animals inoculated or not inoculated with leukemic cells. Removal of the ovary served as a means of demonstrating the participation of that organ's secretions and, conversely, androgen was administered to gonadectomized animals in order to ascertain its effect. In addition, groups of animals under various other natural and artificial conditions with respect to sex hormones were studied.

MATERIAL AND METHODS

The mice used, of the 68th to 73rd inbred generations of strain C58, were 1 month old and 12 to 17 gm. in weight at the start of the experiments. The 30 mice in each of experiments G15, G16, and G17 were divided into 2 groups of 5 males, 2 groups of 5 intact females, and 2 groups of 5 spayed females. In the remaining experiments only spayed females were used: 2 groups of 10 each in G21, and 2 groups of 6 each in G113. The groups were balanced according to weight and litter-origin of the mice within the experiment.

Ovariectomies were performed on the mice under ether anesthesia at one month of age by the method described by Ingle and Griffith (8).

On the day of spaying, hormone pellets were implanted subcutaneously into the animals of one of each pair of groups. With a trocar the pellet was pushed through an incision in the dorsal skin anteriorly into the interscapular region.

The pellets had been prepared for experiments G15, G16, and G17 by dissolving together 2 parts by weight of testosterone propionate[1] and 3 parts of cholesterol in ether; after the solvent had evaporated, the mixture had been compressed manually into cylindrical pellets of 10 to 13 mgm. each. In experiments G21 and G113 the pellets were of pure testosterone propionate and weighed 6 to 7 mgm. each; besides the pellet implanted interscapularly, a second was inserted 30 days later into the inguinal region.

Leukemic cells suspended in saline were inoculated into all mice except those of G113. A dose diluted to 1/256th of the standard of this laboratory was given intraperitoneally in 0.2 cc. to each animal when pellets had been in the treated animals for 21 days in G16, 32 days in G15 and G17, and 60 days in G21. The pellets were not removed at the time of leukemic inoculation.

Line I leukemic cells from transfers 1381, 1380, and 1387 were used for experiments G15, G16, and G17 respectively, and line Ia cells from transfer 1411 were used for experiment G21. Line I had originated in a C58 male of the 17th inbred generation (13), and line

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[1] The testosterone propionate and the cholesterol were gifts from Dr. E. Schwenk and The Schering Corporation to one of us (G. G.).

Ia had been subcultured from line I some 300 transfers before our use. Both lines had been carried almost exclusively in males.

The mice were kept 5 or 6 to a cage and cared for in the manner described by Laanes (11).

The leukemic animals died approximately 6 days after inoculation. Those containing pellets lived a few hours to a day longer than those without extra hormone. When the animals became moribund, the cages were inspected for dead at least each half hour, with the exception of a few longer periods after which animals found dead were discarded. The nonleukemic animals of experiment G113 were killed by bulbar compression on the 66th day after receiving the first pellet, *i.e.*, on the day the leukemic mice of G21 had died. At necropsy, the lymphatic organs were weighed, the treated animals were examined for retention of the pellets, and the complete removal of the ovaries from the spayed females was ascertained.

By accident, hormone pellets were implanted into the control intact females of experiment G16 and not removed until 5 hours had elapsed. However, it appeared that the absorption of hormone in this period was negligible, because the animals behaved with respect to survival time and size of lymphatic organs as though they had had no additional hormone.

Cytological material was prepared as follows: After an inguinal node from each newly dead mouse had been fixed in alcohol-acetic acid 3:1 for several hours, pieces of the specimens were stained in acetocarmine and smeared out under coverglasses. The preparations were dehydrated in 95 per cent alcohol and mounted in diaphane.

The slides were examined under oil immersion in green light by means of an optical system that had a theoretical limit of resolution such that the measurements could be made to the nearest quarter-micron. Ten of the clearer mitotic figures in metaphase were studied at random from preparations of each specimen, and for each mitosis an average chromosome volume was calculated, as previously described (3, 4), to the nearest tenth cubic micron.

Errors in measurement were inevitable, since the chromosomes were usually about 1 μ wide, or 4 to 5 times the limit of resolution, and about 2 μ long. Moreover, in the calculation of average chromosome volume there was a cubing of error in linear measurement. Despite these and other sources of error in the procedure (3), the results bear out the trends apparent to the eye without formal measurements.

TABLE I: MEAN VOLUMES (IN CUBIC MICRONS) OF METAPHASIC CHROMOSOMES FROM INGUINAL NODES OF LEUKEMIC C58 HOSTS WITH OR WITHOUT SUBCUTANEOUS PELLETS CONTAINING TESTOSTERONE PROPIONATE

	Without pellet			With pellet			
Mice	Figs.	Mean volume	St. dev.	Mice	Figs.	Mean volume	St. dev.	Difference between means
			MALES				
G15: 5	50	0.82 ± 0.034	0.236	5	50	0.84 ± 0.032	0.223	0.02 ± 0.047
G16: 5	52	0.90 ± 0.029	0.210	2	20	1.02 ± 0.044	0.196	0.12 ± 0.053
G17: 5	50	0.73 ± 0.023	0.162	5	50	0.80 ± 0.035	0.251	0.07 ± 0.042
			INTACT FEMALES				
G15: 5	50	0.53 ± 0.027	0.188	5	50	0.87 ± 0.029	0.208	0.34 ± 0.040†
G16: 5	50	0.88 ± 0.032	0.228	5	50	0.86 ± 0.033	0.233	—0.02 ± 0.046
G17: 5	50	0.69 ± 0.027	0.191	4	40	0.83 ± 0.028	0.174	0.14 ± 0.039*
			SPAYED FEMALES				
G15: 4	40	0.47 ± 0.027	0.169	5	50	0.76 ± 0.026	0.182	0.29 ± 0.037†
G16: 2	20	0.71 ± 0.028	0.124	2	20	0.97 ± 0.054	0.239	0.26 ± 0.061*
G17: 5	50	0.48 ± 0.023	0.163	5	50	0.81 ± 0.029	0.203	0.33 ± 0.037†

* Significant on 1 per cent level with average chromosome volume per metaphase as unit datum.
† Significant on 1 per cent level with average chromosome volume per metaphase or per animal as unit datum

RESULTS

The average chromosome volumes calculated in experiments G15, G16, and G17 are summarized in Table I. The mean and its standard error, as well as the standard deviation of the distribution of average chromosome volumes, are entered for each set of 20 to 50 drawings made from the groups of 2 to 5 animals. The difference between means of corresponding groups of mice treated and not treated with testosterone propionate is also given, as well as its standard error. The formulas used for these calculations are those given by Arkin and Colton (1).

The comparison of one sex group with another receiving the same treatment was revealing. In the animals given no hormone pellets, there was evidence for a decrease in size of leukemic chromosomes from males through intact females to spayed females: the chromosomes were significantly larger in males than in

intact females in 1 experiment out of 3, in males than in ovariectomized females in all 3 experiments, and in intact than in spayed females in 2 of 3 experiments. Testosterone propionate tended to maintain leukemic chromosomes in spayed and intact females at the size found in males.

The differences between means of corresponding groups with or without exogenous androgen followed a regular pattern. The administration of testosterone propionate was followed by significantly larger chromosomes in spayed females in all 3 experiments, in intact females in 2 experiments, and in males in none. The conflicting result with intact females in one experiment (G16) was occasioned by failure of the females carrying hormone pellets to have larger chromosomes than their controls. All other groups in G16 had chromosomes larger than had the corresponding groups in G15 and G17, perhaps as an age effect, for the animals of G16 were younger than those of G15 and G17.

It was recognized that the inclusion of dividing normal lymphocytes among the figures studied might have been responsible for the small-chromosome metaphases found so abundantly in some specimens. In this case, if the rate of division of normal cells was unchanged, the proportion of small-chromosome figures should have been greater in organs with less leukemic infiltration. According to the data in Table II, the relative leukemic enlargement of spleens was about twice that of inguinal nodes, and yet (Table III) the frequency distributions of average chromosome volumes in inguinal nodes were similar to those in spleens, or even displaced toward larger chromosome sizes than in the spleen (untreated intact females).

TABLE III: FREQUENCY DISTRIBUTIONS OF METAPHASES BY AVERAGE CHROMOSOME VOLUME, AND GROUP MEANS OF CHROMOSOME VOLUME, IN LEUKEMIC INGUINAL NODES (N) AND SPLEENS (S) OF MICE WITH PELLETS (P) OR WITHOUT PELLETS (NO P) CONTAINING TESTOSTERONE PROPIONATE, IN EXPERIMENT G17. TEN METAPHASES FROM NODE AND 6 FROM SPLEEN PER ANIMAL

Av. chrom. vol. (cubic microns)	Number of metaphases											
	Males				Intact females				Spayed females			
	No P		P		No P		P		No P		P	
	N	S	N	S	N	S	N	S	N	S	N	S
0.2-0.3						7			4	6		
0.4-0.5	8	7	9	2	12	15	3	6	34	20	4	5
0.6-0.7	19	7	14	9	19	4	4	13	9	1	13	3
0.8-0.9	21	14	19	16	17	4	28	9	2	3	25	16
1.0-1.1	1	1	1		1		2		1		3	
1.2-1.3	1	1	6	3	1		3	2			5	5
1.4			1									1
Total	50	30	50	30	50	30	40	30	50	30	50	30
Group Means (Cubic microns)	.73	.72	.80	.80	.69	.46	.83	.72	.48	.42	.81	.87

TABLE II: WEIGHTS OF LYMPH NODES AND SPLEENS IN C58 MICE

Mice		Age in weeks	Condition	Leukemia	Average weights of organs (mgm.)		
					Ing. node	All nodes	Spleen
19 females		7	Normal	No			79
5 "		8	"	"	3.0		
13 "		9	"	"			90
3 "		14	"	"			113
6 "	(G113)	14	Spayed	"	6.7	184	158
6 "	"	"	" pellet	"	2.7	57	74
6 "	(G21)	"	Spayed	Yes	14.3	437	747
6 "	"	"	" pellet	"	9.1	225	587
6 males		6½	Normal	No	3.3		93
4 "		14	"	"	4.0		101
3 "		21	"	"	4.4		94
3 "		26	"	"	4.3		
5 "	(G15)	9–10	Intact	Yes	10.2	280	665
5 "	"	"	" pellet	"	8.0	213	723
5 females	"	"	Intact	"	9.2	262	564
5 "	"	"	" pellet	"	8.9	219	635
4 "	"	"	Spayed	"	12.2	374	680
5 "	"	"	" pellet	"	7.3	270	657

A comparison of experiments G21 and G113 (Table IV) gives further indication of the probability that, in females without hormone pellets, we were dealing with leukemic cells containing small chromosomes rather than with normal lymphocytes. The chromosomes in the inguinal nodes of the spayed leukemic females of G21 were significantly larger, on the average, than the chromosomes of nonleukemic lymphocytes of G113. The results in G21 reinforce the observation in the earlier experiments of a greater size of leukemic chromo-

somes in hosts containing testosterone propionate. But the most arresting fact brought out in Table IV is the opposite effect of the hormone on normal and leukemic lymphocytes. Chromosomes of normal lymphocytes were smaller in the animals receiving androgen.

TABLE IV: CHROMOSOME VOLUMES OF LINE Ia LEUKEMIC CELLS AND OF NORMAL LYMPHOCYTES IN INGUINAL NODES OF SPAYED FEMALES WITHOUT PELLETS OF TESTOSTERONE PROPIONATE OR WITH PELLETS FOR 66 DAYS. TEN METAPHASES PER ANIMAL. THE ANIMALS OF G113 ARE PAIRED, THOSE OF G21 ARE NOT

Exper. G21—Leukemics Mean chrom. vol./animal, μ^3		Exper. G113—Nonleukemics Mean chrom. vol/animal, μ^3	
No pellet (I)	Pellet (II)	No pellet (III)	Pellet (IV)
0.46	0.69	0.33	0.15
0.42	0.78	0.33	0.20
0.43	0.71	0.38	0.30
0.43	0.75	0.29	0.18
0.40	0.79	0.34	0.25
0.36	0.74	0.34	0.26
	0.73		

Group means

(I)	(II)	(III)	(IV)
0.42 ± 0.013	0.74 ± 0.022	0.34 ± 0.017	0.22 ± 0.015

Difference between group means

II–I:	0.32 ± 0.026*
III–IV:	0.12 ± 0.023*
I–III:	0.08 ± 0.021*
II–IV:	0.52 ± 0.027*

* Difference is significant on 1 per cent level by "t" test.

Lymphocyte chromosomes were of normal volume in the nonleukemic gonadectomized females without hormone pellets of G113. The mean chromosome volume of 0.34 cubic micron in these spayed females 14 weeks of age did not differ significantly from the value of 0.38 cubic micron in inguinal nodes of normal females 8 weeks old nor from the value of 0.35 cubic micron in mesenteric nodes of normal females at 40 weeks (2).

DISCUSSION

The demonstration that testosterone propionate and perhaps estrogen can influence the size of chromosomes should be of moment in the elucidation not only of chromosomal function in somatic cells but also of the mechanism of the cellular response to hormones. The chromosomal effect is probably but one aspect of the total reaction of the cell. Various authors (5-7, 14) have noted alterations in appearance of nucleus, nucleolus, and chromatin particles in resting nuclei of cells in response to hormonal stimuli. The chromosomes may now also be regarded as members of the system reacting to hormones in sensitive cells.

Why the chromosomes react to androgen is not clear. It might be conjectured that their response is secondary to the known effects of androgen in causing retention of nitrogen (10) or of sodium, potassium, chloride, inorganic phosphorus, and water (9). A dependence of chromosome volume on the concentration of inorganic phosphate in the culture medium has been observed in violet roots by Pierce (15).

The diminution in chromosome size of normal lymphocytes brought about by administration of testosterone propionate may help to explain the reduction in volume of chromosomes in lymphoid tissue occurring over the period of youth and early maturity in C58 mice and in Wistar albino rats (12). Testosterone, or a substance with some properties in common, may then be a factor in the maturation of normal lymphocytes, although apparently lacking effectiveness in this respect when applied to leukemic cells.

The contrasted behavior of the leukemic and the normal chromosomes under treatment by ovariectomy or with testosterone propionate suggests that an attempted explanation involving only hormones and chromosomes might require leukemic chromosomes to differ in make-up from normal. But the possibility of different reaction chains, perhaps involving the cytoplasms, between hormones and chromosomes in leukemic and normal cells must also be entertained. In our particular instance, it is to be borne in mind that androgen had long been a component of the environment of the leukemic cells, because the lines were usually carried in young adult males. Androgen in excess of the customary amount apparently had no effect on leukemic chromosome volume.

SUMMARY

Testosterone propionate implanted in pellets was effective in maintaining the chromosomes of transplanted lines I or Ia leukemic cells in inguinal nodes and spleens of spayed and intact female C58 mice at the large size found in male hosts with or without pellets. In the absence of exogenous androgen, leukemic cell chromosomes in intact female hosts were significantly smaller in 2 of 3 experiments and in ovariectomized female hosts were reduced still further in 4 of 4 experiments toward the size of normal lymphocyte chromosomes in adults.

In contrast, the chromosomes of normal lymphocytes were no larger and perhaps smaller in nonleukemic spayed females bearing hormone pellets than in those bearing none. Ovariectomy alone apparently did not alter the size of normal lymphocyte chromosomes in adult females.

ACKNOWLEDGMENTS

The authors are indebted to Dr. E. C. MacDowell for his constructive criticism and to Misses Jean Gould, Harriet Jackson, and Martha J. Taylor for technical assistance.

REFERENCES

1. ARKIN, H., and COLTON, R. R. An Outline of Statistical Methods. Fourth edition, New York: Barnes & Noble, Inc. 1939, pp. 224.
2. BIESELE, J. J. Chromosomes in Lymphatic Leukemia of C58 Mice. Cancer Research, **7**:70-77. 1947.
3. BIESELE, J. J., and POYNER, H. Polytene Chromosomes in Two Mammary Carcinomas of the Human Subject. Cancer Research, **3**:779-783. 1943.
4. BIESELE, J. J., POYNER, H., and PAINTER, T. S. Nuclear Phenomena in Mouse Cancers. Austin: University of Texas Publication No. 4243. 1942.
5. CLEVELAND, R. Observations on the Chromatin in the Nuclei of Endometrium from Monkeys under Experimental Conditions. Endocrinology, **27**:580-591. 1940.
6. HOOKER, C. W. Effects of Progesterone upon the Uterus of the Mouse. Proc. Soc. Exper. Biol. & Med., **45**:270-272. 1940.
7. HOOKER, C. W. A Criterion of Luteal Activity in the Mouse. Anat. Rec., **93**:333-347. 1945.
8. INGLE, D. J., and GRIFFITH, J. Q. Surgery of the Rat. Ch. 16. The Rat in Laboratory Investigation. edited by J. Q. Griffith and E. J. Farris. Philadelphia: J. B. Lippincott Co. 1942, pp. 376-396.
9. KENYON, A. T., KNOWLTON, KATHRYN, SANDIFORD, IRENE, KOCH, F. C., and LOTWIN, GERTRUDE. A Comparative Study of the Metabolic Effects of Testosterone Propionate in Normal Men and Women and in Eunuchoidism. Endocrinology, **26**:26-45. 1940.
10. KOCHAKIAN, C. D. Testosterone and Testosterone Acetate and the Protein and Energy Metabolism of Castrate Dogs. Endocrinology, **21**:750-755. 1937.
11. LAANES, T. Die Züchtung und Pflege von Laboratoriumsmäusen. Handbuch der biologischen Arbeitsmethoden, edited by E. Abderhalden. Abt. IX, Teil 7. Berlin: Urban & Schwarzenberg. 1936, pp. 593-609.
12. MACDOWELL, E. C., BIESELE, J. J., GASIC, G., TAYLOR, MARTHA J., and LAANES, T. Mouse Leukemia. Carnegie Institution of Washington Year Book No. 44. 1945, pp. 134-139.
13. MACDOWELL, E. C., and RICHTER, M. N. Studies on Mouse Leukemia. V. A Genetic Analysis of Susceptibility to Inoculated Leukemia of Line I. Biol. Zentralbl., **52**:266-279. 1932.
14. PFEIFFER, C. A., and HOOKER, C. W. Responses of the Stromal Nuclei of the Macaque Endometrium to Estrogen and Progesterone. Yale J. Biol. & Med., **17**:249-258. 1944.
15. PIERCE, W. P. The Effect of Phosphorus on Chromosome and Nuclear Volume in a Violet Species. Bull. Torrey Bot. Club, **64**:345-355. 1937.

Chromosomes in Lymphatic Leukemia of C58 Mice

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(Received for publication September 14, 1946)

A larger size of chromosomes in neoplastic cells than in cells of the corresponding normal tissues has been observed in a number of instances (2, 4, 6, 8, 9, 11, 13). Among the several proposed explanations, that of Biesele, Poyner, and Painter (9) suggested that the chromosomes of malignant cells contained multiples of the number of chromosome strands present in those of normal cells. In order to test the hypothesis, studies in lymphatic leukemia of C58 mice have been undertaken. The studies reported here, as well as other observations, indicate that chromosomal enlargement in neoplasms results from more subtle changes than those involved in the multiplication of structural entities near the chromatid level of organization.

MATERIALS AND METHODS

Mice of the C58 strain are noted for high incidence of lymphatic leukemia, about 90 per cent of them dying with the spontaneous disease at some time after the age of 6 months (14). Transmission lines of leukemia are readily obtained (20) and are customarily carried in MacDowell's laboratory in young adult males by means of the intraperitoneal inoculation of suspensions of leukemic spleen in saline.

For the present studies, C58 mice in the 67th to 73rd generations of brother to sister inbreeding were employed. Among them were nonleukemic animals of several ages, young adults inoculated with cells of transplanted leukemic lines, and older animals bearing spontaneous leukemias.

Chromosomes were handled by means of 3 technics: (a) the acetocarmine press method, for a determination of relative sizes; (b) treatment with dilute bicarbonate solutions before fixation and staining with acetocarmine, for an investigation of chromatids, in particular of the number of chromatids per chromosome; and (c) pepsin digestion of fixed and sectioned material, for a determination of relative extents of shrinkage.

(a) In the acetocarmine press method, preparation of the slides after a 2 hour fixation of the specimens in alcohol acetic acid 3:1 was carried out as previously described (8, 9). This method had the advantages of being simple, of maintaining mitotic figures intact, and of yielding chromosomes of larger dimensions than did embedding and sectioning methods. However, histological localization was often rendered impossible and determination of cell type was made difficult by the method. There was no guarantee that all the mitoses studied were of lymphocytes.

An average chromosome volume was calculated for each metaphase figure, as described elsewhere (8, 9), with the aid of camera lucida drawings made at a magnification of about 3,330 times and by means of measurements of chromosome lengths and widths to the nearest quarter-micron. This was possible because the optical system, employing green light with a condenser of N.A. 1.40 and an apochromatic oil-immersion objective of N.A. 1.30, had a theoretical limit of resolution somewhat less than one-fourth micron. The oculars were 20X.

Chromosome sizes in lymphoid tissues were studied in 3 main groups of animals. In the first group, which was made up of nonleukemic mice, the animals used and organs examined were as follows: 4 male and 3 female late embryos, spleen and thymus; 4 male and 3 female newborn animals, spleen and thymus; 9 males 7 or 8 weeks old, spleen, lymph nodes (chiefly mesenteric), and thymus; 5 females about 8 weeks old, spleen and inguinal node; and 1 male and 2 females 37 to 41 weeks old, spleen and mesenteric lymph node.

The second group consisted of 11 females and 1 male with advanced spontaneous cases of lymphatic leukemia. The mice, which were from 34 to 43 weeks old and included littermates of the 3 old nonleukemic animals, were weak and emaciated, and their spleens, lymph nodes, and other infiltrated organs were huge. Acetocarmine preparations of mesenteric nodes from all the animals and of spleens from 4 females were examined.

Animals of the third group bore transplanted leukemic lines. There were 2 or 3 animals of each sex and about 7 weeks old for each line, and the organs studied were variously spleen, liver, mesenteric node, and thymus. Lines I and Ia were near their 1,400th

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transfers, line Mliv near its 1,200th, line L near its 900th, S near its 400th, and U near its 300th. Animals bearing lines I, Ia, or Mliv had been inoculated with one-fourth the standard dose and were sacrificed 3 or 4 days later, shortly before they would otherwise have died. Mice with lines L, S, or U had been inoculated with the standard or one-fourth the standard dose and were killed on the fourth or fifth day.

Finally, 2 new leukemic lines known as V and W were followed from the spontaneous cases through the 25th and 22nd transfers, respectively. The plan was to take specimens of liver and of mesenteric lymph node from 2 animals of each transfer through the 12th and of alternate transfers thereafter. These 2 mice, of which one was usually the donor for the next transfer, were sacrificed about a day after the first death in the transfer group. In some transfers only one animal was taken. Several animals in each transfer were allowed to die from leukemia in order to establish an average interval, or days survived after inoculation. In the preparations from each specimen, 30 metaphases were studied at random. Maximum chromosome width in the figure, measured across both chromatids of chromosomes properly oriented for examination, served instead of average chromosome volume as the unit datum.

(b) A comparison of the chromatids of normal and leukemic lymphocyte chromosomes was made in lymphoid tissue that had been exposed to dilute alkaline solutions. Fresh specimens of nodes and of liver of animals of each sex inoculated with each of the 6 leukemic lines, as well as specimens of lymph nodes of 4 uninoculated controls, were minced with scissors and placed into solutions of 6×10^{-2} to 4×10^{-3} molar sodium bicarbonate at room temperature for 5 to 20 minutes. Small bits were then placed directly into acetocarmine and pressed out for microscopic examination.

(c) Shrinkage of chromosomes by pepsin was studied in 8 experiments involving the partial digestion of sections in acidic solutions of the enzyme. Merck's or Cudahy's commercial pepsin preparations were used in 6 experiments, and crystalline pepsin, kindly provided by Dr. Margaret R. McDonald of this Department, was used in 2 experiments. Normal lymphocytes in an 8 weeks old C58 male were compared with leukemic lymphocytes of line I in brother and sister littermates 8 weeks old. Most study was given to material from 4 newborn males and from 2 groups of 4 littermate males 8 weeks old, 2 of each older group having been inoculated with line Ia leukemia. Various normal and leukemic lymphoid organs were fixed in Carnoy's alcohol acetic acid 3:1 for 2 hours or in a modification of Serra's mixture of alcohol, formalin, and acetic acid (23) in the proportions 12:6:1 for 12 hours and were then dehydrated, embedded in paraffin, and sectioned at 7 microns. The sections mounted on slides were placed into a solution of 0.1 per cent pepsin and 0.2 per cent HCl at 37° C. ± 1° for one to 22 hours, and control sections were incubated under the same conditions in a 0.2 per cent HCl solution lacking pepsin. After incubation, control and experimental slides were placed together and given the Feulgen test for desoxyribose nucleic acid, with or without light green counterstaining, or were stained in aqueous toluidine blue. Digestion of cytoplasmic protein by pepsin was indicated by failure of the cytoplasmic areas to be counterstained. Attempts were made to measure the lengths of individual chromosomes in early experiments, but because of the small size of the chromosomes after pepsin digestion, measurements of the maximum diameters of metaphase figures were resorted to in the experiments with crystalline pepsin for more conclusive data.

RESULTS

The determination of chromosome volume in acetocarmine smears revealed a variation with age in normal lymphoid tissue (Fig. 1). From an intermediate value in the late embryo, the mean chromosome volume increased somewhat in the newborn animals but decreased significantly through those near 2 months old to a low value in the mature animals near 40 weeks of age. Chromosome sizes were larger in the spleen than in the thymus, and they were intermediate or about equal to the splenic values in the lymph nodes.

Chromosomes in the 12 spontaneous cases of leukemia were significantly larger than those in normal lymphoid tissue of animals of the same age, but they were of about the same size as normal lymphocyte chromosomes in younger animals 2 months old. Chromosomes in the one male with spontaneous leukemia agreed in size with those of the females with spontaneous cases.

In the long-transplanted lines, however, the leukemic cell chromosomes were considerably larger than in the spontaneous cases, and there was a sex difference. While chromosomes of all 6 leukemic lines in female hosts were equivalent in size to the chromosomes of normal spleen in newborn animals, the leukemic cell chromosomes in male hosts attained mean volumes greater than any reached during ontogeny by the normal lymphocytes, so far as studied. The range in chromosome volume in leukemic tissues extended downward to overlap much of the range of normal chromosome volume. There was no appreciable difference in size of chromosomes between the 3 older and more virulent lines I, Ia, and Mliv, and the 3 younger lines, L, S, and U.

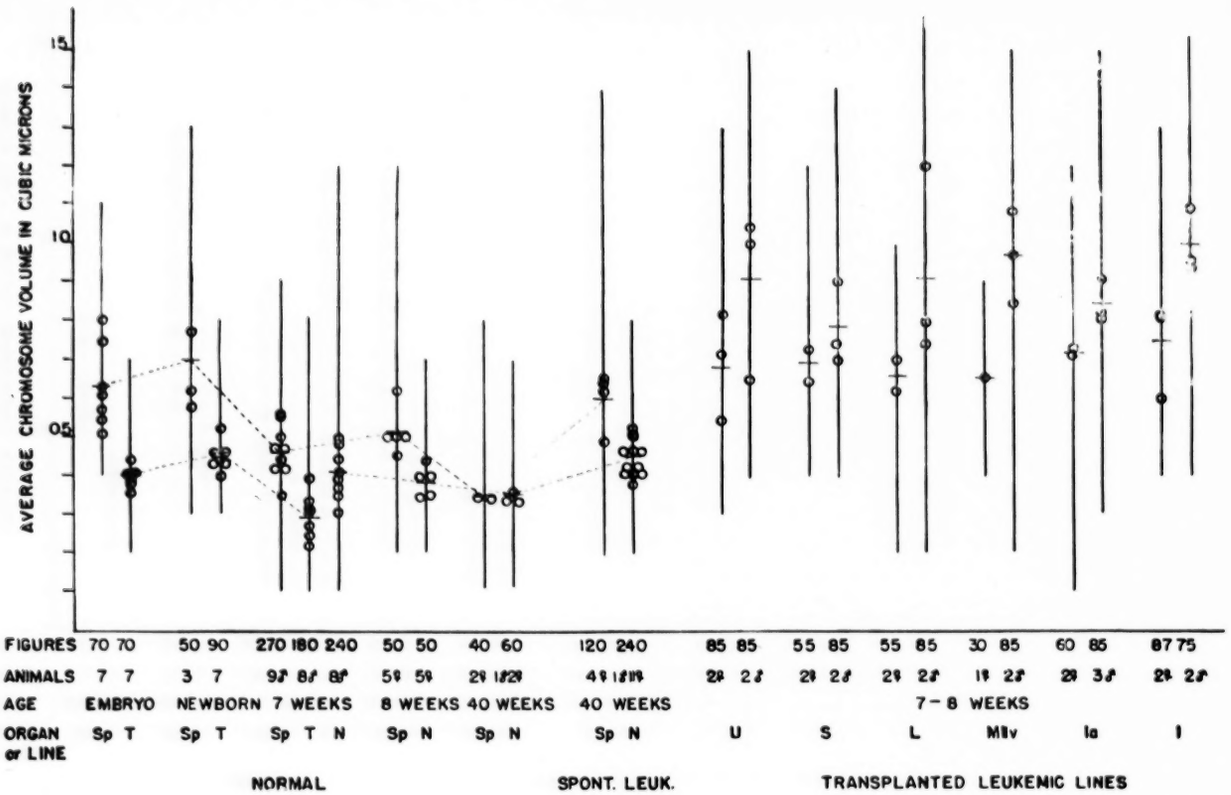
AVERAGE CHROMOSOME VOLUME IN CUBIC MICRONS
FIGURES 70 70 50 90 270 180 240 50 50 40 60 120 240 85 85 55 85 55 85 30 85 60 85 87 75
ANIMALS 7 7 3 7 9♀ 8♂ 8♀ 5♀ 5♀ 2♀ 1♂2♀ 4♀ 1♂1♀ 2♀ 2♂ 2♀ 2♂ 2♀ 2♂ 1♀ 2♂ 2♀ 3♂ 2♀ 2♂
AGE EMBRYO NEWBORN 7 WEEKS 8 WEEKS 40 WEEKS 40 WEEKS 7–8 WEEKS
ORGAN or LINE Sp T Sp T Sp T N Sp N Sp N Sp N U S L Mlv Ia I
NORMAL
SPONT. LEUK.
TRANSPLANTED LEUKEMIC LINES

FIG. 1

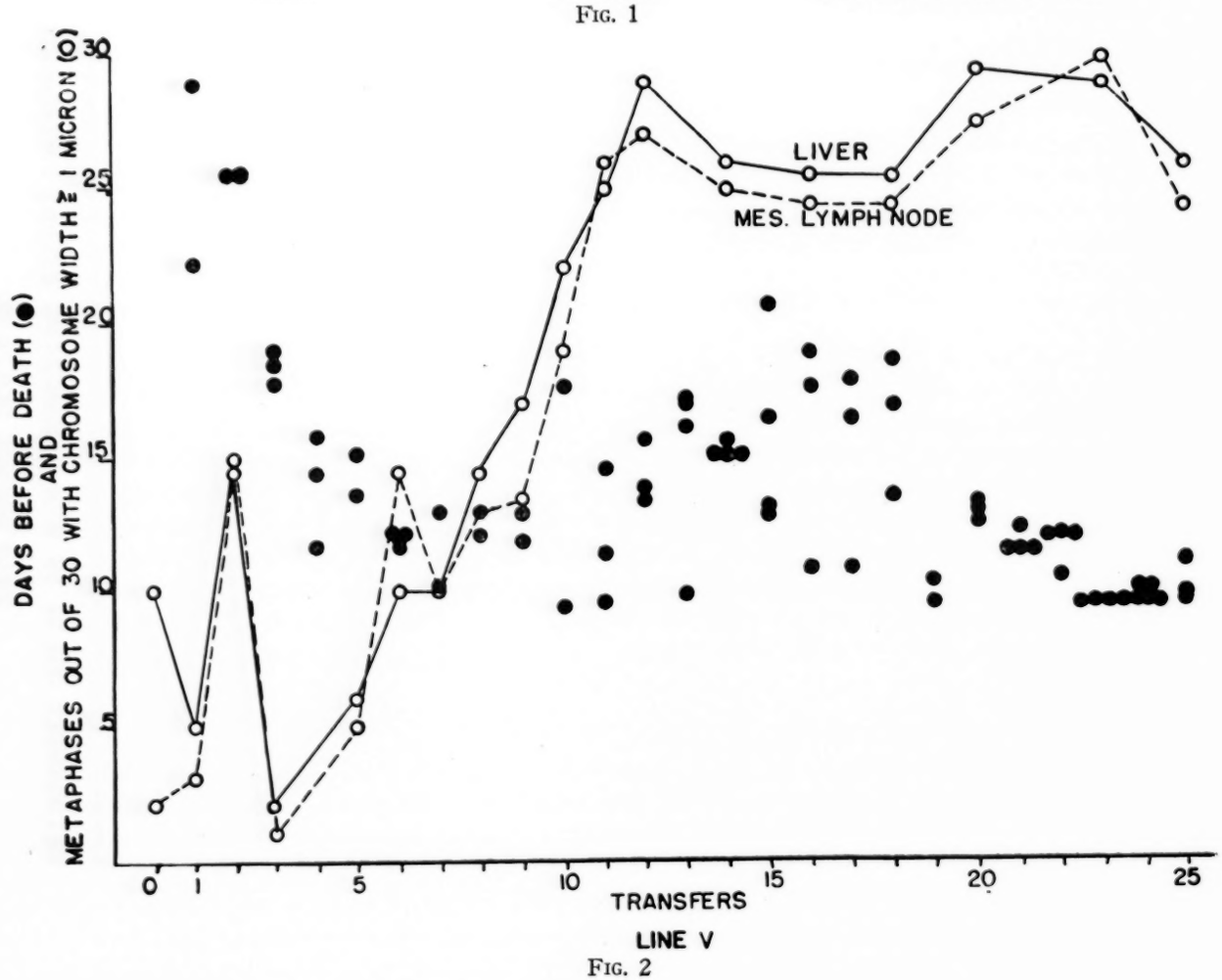
DAYS BEFORE DEATH (●)
AND
METAPHASES OUT OF 30 WITH CHROMOSOME WIDTH ≥ 1 MICRON (○)
LIVER
MES. LYMPH NODE
TRANSFERS
LINE V

FIG. 2

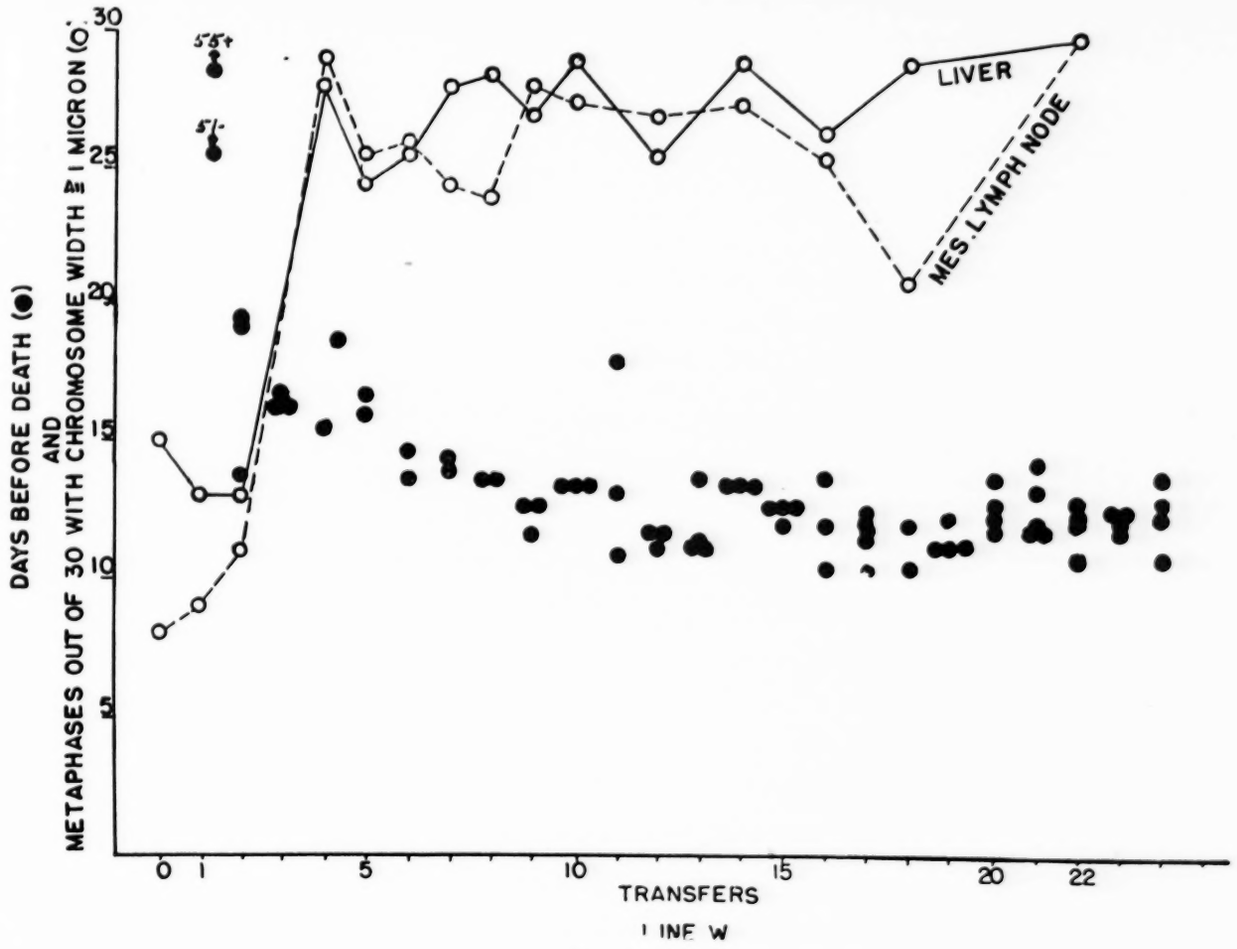

FIG. 3

Figs. 4, 5, and 6 illustrate lymphocyte chromosomes from a normal young adult male, from a case of spontaneous leukemia, and from line I in a male host respectively.

The transition in chromosome size from the spontaneous cases to the long-transferred lines was studied in the 2 new lines, V and W. As Figs. 2 and 3 show, the proportion of metaphases with large chromosomes a micron or more in width rose gradually from the spontaneous case's low value, which was slightly greater than normal, to become stabilized at a high value by the 11th or 12th transfer in line V, while the rise was more abrupt in line W, having been accomplished between the second and fourth transfers. The high proportion of metaphases with large chromosomes, which was reached relatively early in V and W, was also characteristic of the long-transferred lines.

Changes in interval, or the days between leukemic inoculation and death, followed similar but not identical courses in V and W. In both there was a rapid drop from the long interval in the first transfer over successively shorter intervals in the next 4 to 10 transfers to

DESCRIPTION OF FIGURES 1 TO 3

FIG. 1.—Average metaphasic chromosome volumes in normal and leukemic lymphoid tissues of C58 mice. Vertical lines represent ranges; horizontal lines, group means; and circles, means for individual animals or organs (in leukemics). The dash lines connect group means for given organs. Sp = spleen, T = thymus, and N = lymph nodes. U, S, L, Mliv, Ia, and I are leukemic lines. "Figures 70 70 50 90" etc. denote numbers of metaphase figures.

FIG. 2.—Relative frequency of metaphases with large chromosomes (averaged from 2 animals of each transfer) and interval from inoculation to death of individual animals in early transfers of line V.

FIG. 3.—Relative frequency of metaphases with large chromosomes (averaged from 2 animals of each transfer) and interval from inoculation to death of individual animals in early transfers of line W.

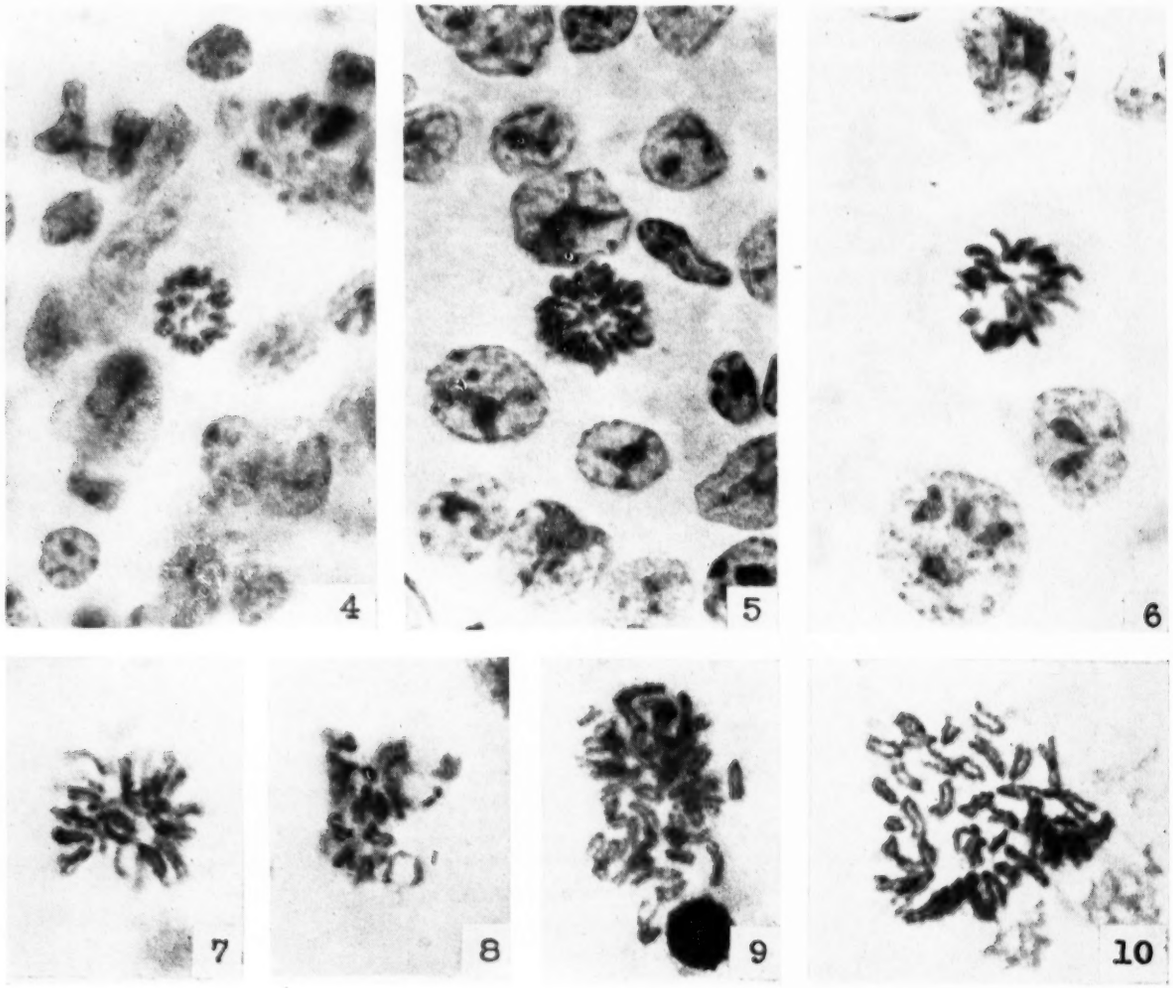

FIG. 4.—Nonleukemic metaphase figure in spleen of normal male C58 mouse 7 weeks old. Acetocarmine press. Mag. × 1,200.

FIG. 5.—Metaphase figure from infiltrated mesenteric lymph node of 10 months old female C58 mouse with spontaneous leukemia. Acetocarmine press. Mag. × 1,200.

FIG. 6.—Leukemic cell mitosis from liver of male C58 mouse inoculated with line I three days earlier. Acetocarmine press. Mag. × 1,200.

FIGS. 7 and 8.—After sodium bicarbonate treatment, mitotic figures in normal mesenteric nodes of nonleukemic male C58 two months old. Acetocarmine press. Mag. × 1,300.

FIG. 9.—After sodium bicarbonate treatment, leukemic cell mitosis in mesenteric node of female C58 two months old inoculated with line S. Acetocarmine press. Mag. × 1,300.

FIG. 10.—After sodium bicarbonate treatment, leukemic cell mitosis in liver of male C58 two months old inoculated with line I. Acetocarmine press. Mag. × 1,300.

a period of very slow decrease in interval. Single animals in given transfers agreed well with one another in line W, but in line V transfers 10 to 18 were marked by considerable scatter in interval.

The relation between the interval before death and the proportion of metaphases with large chromosomes in V and W was not clear. While the approximate stabilization of minimum interval and the leveling-off of large chromosome metaphases at a high proportion occurred over much the same number of early transfers in V, they did not agree in timing in W. Chromosome size in these 2 lines had come to be equivalent to that in the older lines, but V and W still faced a probable reduction of one-half in interval, according to the history of other lines of leukemia in C58 mice.

In leukemic cells of all 6 lines metaphasic chromosomes uncoiled by dilute alkaline solutions and then both fixed and stained in acetocarmine appeared to differ from normal only in their larger size. The chromosomes were composed of 2 loosely coiled chromatids, which were often well separated and coherent only at the centromere (Figs. 7 to 10). No internal differentiation was evident in the chromatids, and those in leukemic cell chromosomes were not structurally double the normal, so far as could be determined.

TABLE I: AVERAGE CHROMOSOME LENGTHS IN SECTIONED NORMAL AND LEUKEMIC TISSUES AFTER INCUBATION IN PEPSIN OR CONTROL SOLUTIONS

	Lengths after HCl alone			Lengths after HCl + pepsin		
	Group mean	Figure means		Group mean	Figure means	
Lymphocytes		Range	No.		Range	No.
Normal in male	1.2μ	1.2-1.3μ	6	1.0μ	0.8-1.3μ	14
Leukemic in male	1.6	1.4-1.7	6	1.0	0.9-1.1	6
Leukemic in female	1.5	1.3-1.6	10	1.0	0.9-1.1	13

The essential result of the experiments with pepsin digestion was that the leukemic chromosomes were shrunken proportionally more than the normal lymphocyte chromosomes, and that much if not all of the difference in volume between normal and leukemic chromosomes was thus abolished. A few observations on chromosome length in material stained by Feulgen's method are recorded in Table I. Although they are necessarily of questionable accuracy, they are in harmony with more reliable measurements, which were made of the maximum diameters of metaphase figures in the tests with crystalline pepsin. The results of the latter measurements appear in Table II. The diameters are given in units equal to 0.3 micron each and are pooled from observations on material treated for 1, 3, or 5 hours, since changes occurring after 1 hour appeared insignificant. Contraction of chromosomes in lymphoid tissue of newborn males was intermediate in extent. Execution of the Feulgen reaction after treatment with pepsin or incubation in the control solution appears to have introduced its own complicating shrinkage. The least contraction after pepsin digestion occurred in material fixed in Serra's fluid.

TABLE II: MAXIMUM DIAMETERS OF METAPHASE FIGURES IN NORMAL AND LEUKEMIC LYMPHOID TISSUES OF C58 MALES AFTER INCUBATION IN HCL SOLUTIONS WITH OR WITHOUT PEPSIN

	Control figures			Pepsin figures			
	Maximum diameters			Maximum diameters			Linear shrinkage
	Average	Range	No.	Average	Range	No.	(%)
Carnoy-fixed; Feulgen:							
Norm. in 2-mo. spleen	16.2	12-23	80	13.7	9-16	50	15
Norm. in 2-mo. node.	16.6	11-24	140	11.8	9-18	115	29
Norm. in 2-mo. thymus	16.0	11-21	75	11.7	9-16	75	27
Mean	16.3			12.4			24
Norm. in newborn spleen	18.1	15-24	60	14.0	10-20	57	23
Norm. in newborn thymus	17.5	13-25	45	12.1	10-15	45	31
Mean	17.8			13.1			27
Leuk. in 2-mo. spleen	22.7	18-30	150	14.3	8-22	150	37
Leuk. in 2-mo. node	22.5	16-31	150	13.7	10-24	125	39
Mean	22.6			14.0			38
Carnoy-fixed; toluidine blue:							
Norm. in 2-mo. node	18.7	14-32	65	16.0	12-21	55	14
Norm. in newborn spleen	22.5	17-30	60	16.2	12-20	60	28
Leuk. in 2-mo. spleen	24.2	17-29	90	17.7	13-25	85	27
Serra-fixed; toluidine blue:							
Norm. in 2-mo. node	17.0	13-20	40	16.1	12-24	40	5
Leuk. in 2-mo. spleen	20.3	17-23	40	18.1	13-22	40	11

NOTE: The diameters are given in units equal to 0.3 μ each.

DISCUSSION

The shrinkage of chromosomes brought about by pepsin has been observed by several authors (15, 17, 18, 21). Mazia (17) has ventured the explanation that the pepsin was able to digest some protein constituent of the chromosomes, possibly an acidic "matrix" protein, the removal of which allowed a further contraction of the remaining "skeletal" constituents of the chromosomes. When fibers formed from mixtures of histone and the "nucleoprotein X" of Huiskamp (12) were exposed to pepsin by Mazia (17), the latter component of the fibers was digested immediately and the rest of the fibers, apparently continuous chains of histone, became contracted; fibers of histone or of nucleohistone were not broken down by the enzyme. Mazia therefore suggested that the continuous structure of the chromosome was made up of histone-like protein. Of the components of chromosomes found by Mirsky, Pollister, and Ris (19), the complex tryptophane-containing protein would seem more likely to be digested by pepsin than would desoxyribose nucleic acid as such or the basic proteins, histone and protamine. Their tryptophane-containing protein has points of resemblance to the protein, chromosomin, which Stedman (24, 25) has claimed to be the predominant constituent of chromosomes and to be present in especially high concentration in isolated nuclei of carcinomas and of embryonic chicken tissue.

A smaller size of chromosomes in material subjected

to the Feulgen reaction than of those in tissue stained with toluidine blue might be expected if the hydrolysis in the Feulgen procedure removed some of the protein, as has been claimed for histone by Stedman (24). Not only digestion of the non-histone protein, but also contraction of the histone remaining, might be impaired by Serra's fixative because of its content of formaldehyde, which is known to react chemically with amino groups and amido nitrogen of proteins (27).

The shrinking of lymphocyte chromosomes exposed to pepsin may be tentatively attributed to the digestion of protein or proteins equivalent to Mazia's protein of the chromosome matrix, Mirsky's tryptophane-containing protein, or Stedman's chromosomin. Different quantities of this protein material may then be the immediate cause of the difference in volume between the normal and the leukemic lymphocyte chromosomes, inasmuch as they shrink to about the same small size under pepsin treatment. If so, there may be no need to postulate a greater number of strands in cancer chromosomes in order to explain their increased volume; the hypothesis of Biesele, Poyner, and Painter (9) to this effect may be untenable, and the author's other papers (1-4, 6, 8) on chromosome size in neoplasms may require a revised interpretation.

Since the tryptophane-containing protein of Mirsky is combined with nucleic acid in the chromosomes (19), increase in the protein probably means an increase in nucleic acid. If the pepsin-digestible substance of leukemic chromosomes is tryptophane-containing protein, a modification may be required in the assumption of Thomas (26) that the hyperchromatism of cancers, in the sense of enlarged chromosomes and greater staining capacity, results solely from the presence of additional nucleic acid in the chromosomes.

Variations in content of pepsin-digestible protein are not to be considered only in the comparison of normal chromosomes with those of neoplastic cells, however. In the first place, Mirsky, Pollister, and Ris (19) found different proportions of tryptophane-containing protein in the isolated chromosomes of various normal cell types, and in the second place, as we have seen in Table II, the chromosomes of lymphoid tissue in newborn mice seemed to shrink more under pepsin treatment than did those in young adults. There is hence the possibility that variations in normal lymphocyte chromosome size during the development of the mouse, which were paralleled in lymphoid tissues of rats (5), also depend largely on variations in quantity of pepsin-digestible protein. However, the possibility of differences in amounts of other constituents as well is not to be denied. In this connection, the discovery of a considerable amount of firmly-bound lipoid in chromosomes isolated from rat liver has been reported by Marshak and Walker (16).

The variability in amounts of particular chemical components and hence in volume of chromosomes from one cell to another is probably related to differences in cellular function. Ris (22) has recently stated that the gene is to be regarded as a complex whose volume may vary widely and thus give rise to nonuniformity of chromonema length in cells differing in metabolic activity. In the study of the variation of normal rat chromosome size with organ (cell type) and age (5), it was concluded that changes in chromosome size were most likely to occur over periods during which cell differentiation or modulation took place in the tissue concerned; the differentiating intermitotics (10) showed the most alterations in chromosome volume. The morphological responses of lymphocyte chromosomes to ovariectomy and testosterone propionate (7) might be considered as subsidiary aspects of the same general phenomenon. Finally, the changes in chromosome volume occurring in the transition from normal cell to malignant cell may well be viewed in this same light.

SUMMARY

Chromosomes were found to enlarge progressively from normal lymphocytes in adult C58 mice through the lymphocytes of spontaneous leukemias and of the early transfers of leukemic lines to the highly virulent leukemic cells of long-transferred lines. This overall 2-fold or 3-fold volume increase of the average metaphasic chromosome in leukemia reversed and overcompensated for the normal ontogenetic reduction of about one-half in lymphocyte chromosome size from the newborn animal to the adult.

Uncoiling of chromosomes in dilute alkaline solution revealed no difference in microscopic complexity of structure between leukemic cell chromosomes and normal lymphocyte chromosomes.

Partial digestion of sections by pepsin shrank the chromosomes of leukemic cells relatively more than normal lymphocyte chromosomes and thus eliminated most of the size difference between them. If shrinkage occurred because of the removal of pepsin-digestible protein and the contraction of the residual nucleohistone, the differences in chromosome size between normal and leukemic lymphocytes might be attributed largely to difference in amounts of pepsin-digestible protein.

ACKNOWLEDGMENTS

The author is indebted to Dr. E. C. MacDowell for helpful advice and criticism, to Mr. M. G. Schultz for preparation of the photomicrographs, and to Misses Martha J. Taylor, Harriet Jackson, and Jean Gould for technical assistance.

REFERENCES

1. Biesele, J. J. Diplochromosomes in a Goldfish Tumor. Cancer Research, **3**:411-412. 1943.
2. Biesele, J. J. Size and Synthetic Activity of the Chromosomes of Two Rat Neoplasms. Cancer Research, **4**:540-546. 1944.
3. Biesele, J. J. Ribonucleic Acid and Heterochromatin in Epidermal Carcinogenesis. Cancer Research, **4**: 737-750. 1944.
4. Biesele, J. J. Chromosomal Enlargement in Neoplastic Rabbit Tissues. Cancer Research, **5**:179-182. 1945.
5. Biesele, J. J. The Size of Somatic Chromosomes at Different Ages in the Rat. J. Gerontology (in press).
6. Biesele, J. J., and Cowdry, E. V. Chromosomal Changes in Epidermal Carcinogenesis. J. Nat. Cancer Inst., **4**:373-384. 1944.
7. Biesele, J. J., and Gasic, G. Sex Hormone Effects on Chromosome Size in Leukemic and Normal Lymphocytes of C58 Mice. Cancer Research, **7**: 65-69. 1947.
8. Biesele, J. J., and Poyner, H. Polytene Chromosomes in Two Mammary Carcinomas of the Human Subject. Cancer Research, **3**:779-783. 1943.
9. Biesele, J. J., Poyner, H., and Painter, T. S. Nuclear Phenomena in Mouse Cancers. Austin: University of Texas Publication No. 4243. 1942.
10. Cowdry, E. V. Ageing of Individual Cells. Ch. 24, Problems of Ageing, 2d Edition. Baltimore: The Williams & Wilkins Co. 1942, pp. 626-663.
11. Goldschmidt, R., and Fischer, A. Chromosomenstudien an Carcinomzellen in vitro. Ztschr. f. Krebsforsch., **30**:281-285. 1929.
12. Huiskamp, W. Beiträge zur Kenntnis des Thymusnucleohistons. Ztschr. f. physiol. Chem., **39**:55-72. 1903. (17).
13. Ludford, R. J. The Behaviour of the Malignant and Non-Malignant Cells of Transplantable Tumours in Tissue Cultures. Arch. f. exper. Zellforsch., **14**:42-55. 1933.
14. MacDowell, E. C., and Richter, M. N. Mouse Leukemia. IX. The Rôle of Heredity in Spontaneous Cases. Arch. Path., **20**:709-724. 1935.
15. Maneval, K. Chemical Structure of Plant Chromosomes. M. A. Thesis, University of Missouri. Cited by Mazia, (17).
16. Marshak, A., and Walker, A. C. Effect of Liver Fractions on Mitosis in Regenerating Liver. Am. J. Physiol., **143**:226-234. 1945.
17. Mazia, D. Enzyme Studies on Chromosomes. Cold Spring Harbor Symposia on Quant. Biol., **9**:40-46. 1941.
18. Mazia, D., and Jaeger, Lucena. Nuclease Action, Protease Action and Histochemical Tests on Salivary Chromosomes of Drosophila. Proc. Nat. Acad. Sc., **25**:456-461. 1939.
19. Mirsky, A. E., Pollister, A. W., and Ris, H. The Chemical Composition of Chromosomes. Genetics, **31**:224-225. 1946.
20. Richter, M. N., and MacDowell, E. C. Studies on Leukemia in Mice. I. The Experimental Transmission of Leukemia. J. Exper. Med., **51**:659-673. 1930.
21. Ris, H. A Cytological and Experimental Analysis of the Meiotic Behavior of the Univalent X Chromosome in the Bearberry Aphid Tamalia (= Phyllaphis) cowenii (Ckll.). J. Exper. Zool., **90**:267-330. 1942.
22. Ris, H. The Structure of Meiotic Chromosomes in the Grasshopper and its Bearing on the Nature of "Chromomeres" and "Lamp-brush Chromosomes." Biol. Bull., **89**:242-257. 1945.
23. Serra, J. A. Histochemical Tests for Proteins and Amino Acids; the Characterization of Basic Proteins. Stain Technol., **21**:5-18. 1946.
24. Stedman, E. The Chemistry of Cell Nuclei. (Honyman Gillespie Lecture.) Edinburgh Med. J., **51**:353-366. 1944.
25. Stedman, E. The Chemistry of Cell Nuclei. Biochem. J., **39**:lviii-lix. 1945.
26. Thomas, P. T. Experimental Imitation of Tumour Conditions. Nature, London, **156**:738-740. 1945.
27. Walker, J. F. Formaldehyde. Am. Chem. Soc. Monograph No. 98. New York: Reinhold Publishing Co. 1944, pp. 397.

Stromal Malignancy in Mouse-Grown Transplants of Egg-Cultivated Mouse Mammary Carcinoma

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(Received for publication May 13, 1946)

Several papers have been published from this laboratory giving the results of investigations designed to demonstrate tumor production in mice with cell-free extracts of materials from eggs containing implants of mouse mammary carcinoma. Successful experiments with both Berkefeld filtered and lyophilized yolk from eggs containing yolk sac implanted mouse mammary tumor have been reported (6, 10, 11). It has also been shown that mouse tumor tissue inoculated into the anterior chamber of the rat's eye may induce malignancy in cells of rat origin (9, 10).

While these investigations have furnished evidence for the tumor agent or virus concept of cancer causation, the methods used cannot be depended upon to demonstrate regularly the presence of the tumor agent in the various extracts under investigation. Many hundreds of experiments have been entirely negative and other laboratories which have as far as possible followed the procedures used here have failed to obtain positive results (1, 3, 13).

The conclusion, however, of Twombly and Meisel (13) that such tumors as have been produced from the various extracts of tumor-bearing eggs were due to the presence in the material injected of viable tumor cells is not tenable. The 20 malignant tumors obtained from lyophilized extracts cannot be explained on this basis since the material injected, not only failed to give tumors subsequently to the successful experiment, but also in previous attempts. This alone rules out the presence of tumor cells since the tumor concerned in this work "takes" in 100 per cent of the mice injected. Further, all 20 of the tumors produced by the lyophilized material were sarcomas in contrast to the donor mammary carcinoma and remained stable through numerous transplants, never in any instance assuming a structure which resembled a mammary carcinoma. In addition, these new tumors were characterized by the presence of giant cells, nuclear debris, and unusual mitotic aberrations, none of which were observed in the donor tumor.

It is understandable that where attempts to duplicate experiments of this nature are unsuccessful, workers in other laboratories become skeptical of the validity of the observations reported. But it must be kept in mind that an agent may be involved which is demonstrable only under very special conditions. The experience here indicates an almost all-or-none quality in the activity of the tumor-producing principle.

Recently Duran-Reynals (2) reported that the age of the chickens used to grow the donor tissue influenced the adaptation of the tumor virus to duck tissue. This is an example of how sensitive a recognized tumor virus is to just one factor of its environment.

The present paper is concerned with a different approach to what is believed to be the same phenomena of tumor induction by a tumor agent. Unlike the previous reports, the method involved gives results which are repeatable.

In association with the study of methods for demonstrating the presence of a tumor-inducing agent in tumor tissue, the technic of cultivating cancer tissue in eggs by the yolk sac method has been perfected (8, 12). Mouse mammary carcinoma has been carried in eggs continuously in this manner for the past 2 years and 11 months.

It has been previously noted that extracts of yolk material from tumor-bearing eggs quite regularly produce tumors when injected into mice. This was not surprising since there were reasons for believing these extracts contained tumor cells. However, in some instances the tumors produced in this manner differed in cell origin from the donor mammary carcinoma (11). More recently it has been found that when tumor tissue from the egg-grown series was injected into mice the resulting tumor was frequently a mixed carcinoma-sarcoma. Since the egg-cultivated tumor has always remained histologically stable, transplants into mice can be made with the same basic material and the induction of malignancy in the normal cells of the stroma studied for long periods and under various conditions.

Instances of sarcomatous transformation of the stroma of mouse transplants have been reported. Recently Ludford and Barlow (4) reviewed the literature and described this phenomenon in a group of mouse carcinomas maintained by mouse transplants.

MATERIALS AND METHODS

Tumor tissue was obtained from egg-cultivated mouse mammary carcinoma. In most experiments the tumor tissue used had been kept continuously in eggs for 30 to 70 transplant-generations. A few experiments were carried out with tumor tissue grown in eggs for one or a few transplant-generations.

The mouse mammary carcinoma used in these experiments occurred spontaneously in breeder stock in 1941 and has since been designated as dba mammary carcinoma 1 (6). During the period of approximately 2 years it was carried in mice, and for the 2 years and 11 months it has been kept continuously in eggs, this tumor has maintained the histological appearance typical of a fast-growing homogenous mammary carcinoma.

The egg series is maintained by the yolk sac method essentially as previously described (8, 12). More experience with this technic has rendered it a simple and effective means for the continuous production of tumors suitable for egg cultivation. Yolk-sac-grown dba carcinomas average 1 to 2 gm. 13 days after inoculation. Since the blood and stroma are supplied by the chick, the mouse cancer tissue is contiguous to normal cells of chick origin only. This probably accounts for the stability of the egg-grown tumors.

Male and female dba mice 3 to 4 months old were used for carrying the transplants in mice. Inoculation was made subdermally into 2 or 3 mice by hypodermatic injection of a 1:4 saline suspension of tumor tissue. Transplants grown in mice were harvested and re-implanted after a period of 15 to 20 days at which time the tumors averaged 3 to 4 gm. Tumor tissue from each transplant-generation was prepared for histological study. The various sections of tumor tissue were evaluated with regard to the cytological characteristics and extent of any sarcomatous tissue which might be present.

The data presented are based on the results obtained in a series of 186 experiments involving the use of 2,440 mice.

RESULTS

Data are given in Tables I and II and Figs. 1 to 10. Sarcomatous cells were first observed in isolated patches in the stroma of the transplant. Later when the proportion of new tumor to original mammary tumor had increased, the separate groups became linked together. Histological sections at this stage presented irregular masses of mammary tumor enclosed by narrow strips of sarcomatous tissue (Figs. 2 and 9).

TABLE I: IMPLANTS IN MICE OF EGG-GROWN MAMMARY CARCINOMA. PER CENT OF TRANSPLANTS CONTAINING AREAS OF SARCOMATOUS TISSUE OR COMPLETE REPLACEMENT OF THE ORIGINAL TUMOR FOR EACH TRANSPLANT-GENERATION

Transplant-generation in mice	No. of exper.	No. of mice	No. of exper. showing areas of sarcoma	Per cent exper. showing areas of sarcoma
1	186	547	71	38.1
2	186	366	124	66.7
3	180	362	125	69.4
4	178	361	125	70.2
5	145	292	109	75.2
6	104	212	77	74.0
7	62	128	36	58.1
8	42	88	27	64.3
9	23	50	13	56.5
10	15	34	10	66.7

The mammary tumor used in these experiments has a very limited stroma, and there was no observable tendency for stromal hyperplasia to develop prior to the appearance of sarcomatous tissue.

Successive generations of mouse transplants tended toward increase in the amount of new tumor present. In some instances, however, after more than 30 per cent

TABLE II: LIST OF EGG-GROWN MAMMARY CARCINOMAS COMPLETELY REPLACED BY SARCOMAS

Exper. no.	Total no. transplant-generations in mice	Transplant-generation showing areas of sarcoma	Transplant-generation showing complete replacement by sarcoma	Description of sarcoma
1	2	1	2	Round-cell sarcoma with nuclear debris
2	45	1	2	(Not classified) with nuclear debris
3	5	1	4	Spindle-cell sarcoma
43	6	3	6	Spindle-cell sarcoma
46	9	2	8	Spindle-cell sarcoma with nuclear debris
58	7	2	5	(Not classified) with nuclear debris, giant cells, mitotic aberrations
66	13	7	9	Spindle-cell sarcoma
67	11	3	5	Spindle-cell sarcoma
113	19	1	6	Lymphosarcoma
114	15	1	10	Unclassified sarcoma
115	15	2	13	Unclassified sarcoma

of the tumor was of the sarcoma type, it reverted to the original mammary carcinoma in subsequent transplants. The ultimate status of the new tumor in relation to the original carcinoma could be evaluated to some extent by its relative rate of cell division. When the newly induced tumor had a relatively high rate of division, it quickly replaced the original mammary tumor. In experiments 1 and 2, the second mouse transplant-generation was made up entirely of new tumor (Fig. 5). This tumor has been carried through more than 45 generations of transplants and has retained the characteristics it had in its first appearance. It is an exceedingly fast-growing tumor, hence its quick replacement of the original mammary tumor.

When the growth rate of the new tumor was relatively low, sections of transplants showed a mixed tumor through many months of transplant propagation.

Giant cells of various types and unusual mitotic aberrations were common in most of the new tumor tissue. In many instances a certain proportion of the cells were unable to complete division. The chromatin material became condensed in heavy masses and this seemed to account for most of the nuclear debris so characteristic of some of the new tumors (Figs. 3, 5).

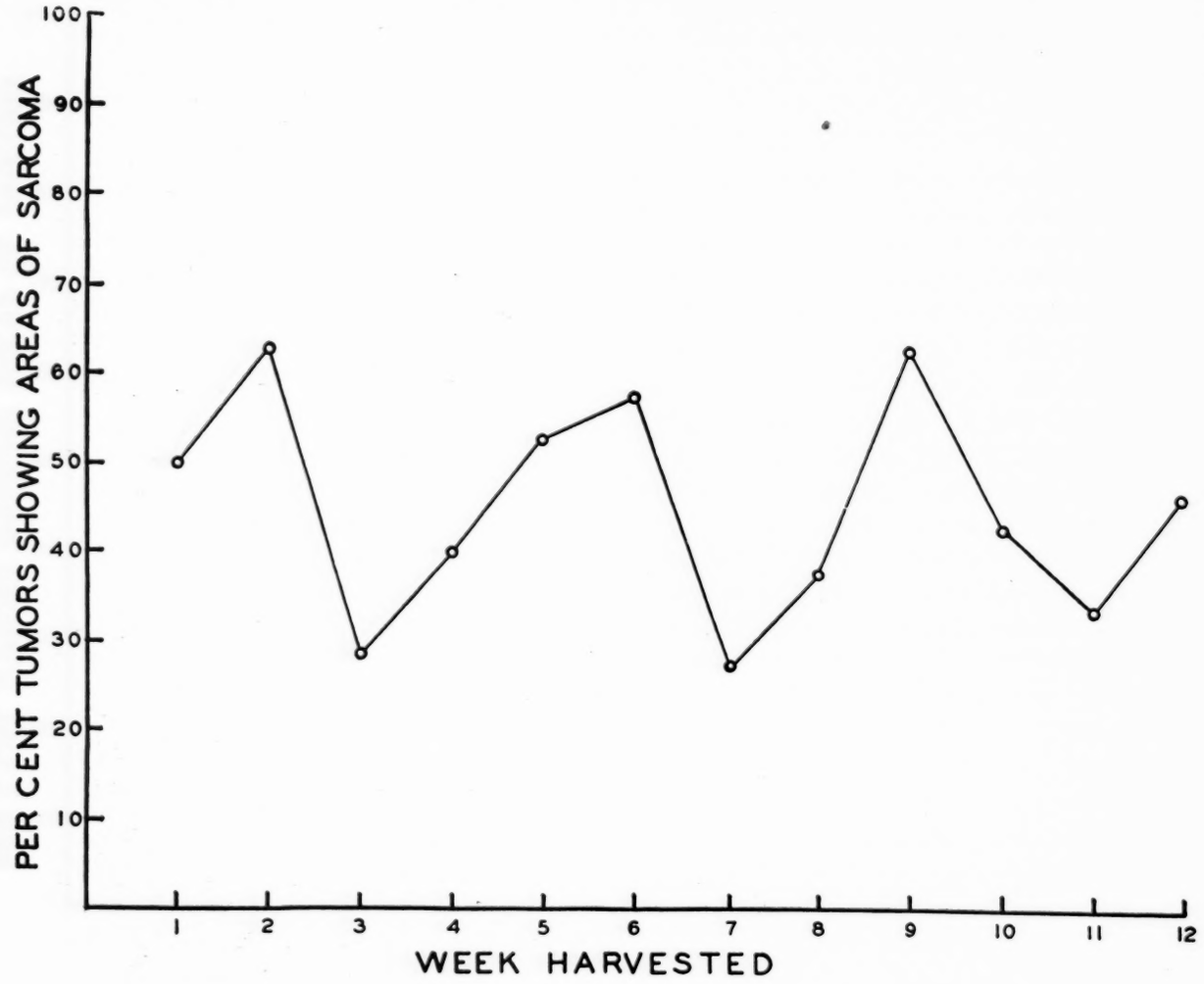


FIG. 1.—Variation in activity of tumor agent over a period of 12 weeks, January 7 through March 31, 1946, based on a series of 341 mice.

In many experiments, however, the new tumor presented the appearance of a fast-growing tissue in which no unusual cell aberrations appeared. Giant cells and nuclear debris were not present in the host mammary tumor. (Fig. 7).

Centrifugation of suspensions of mammary tumor tissue which contained areas of sarcomatous tissue resulted in two layers—the upper layer contained the sarcomatous cells.

No effort has been made to make more than a very

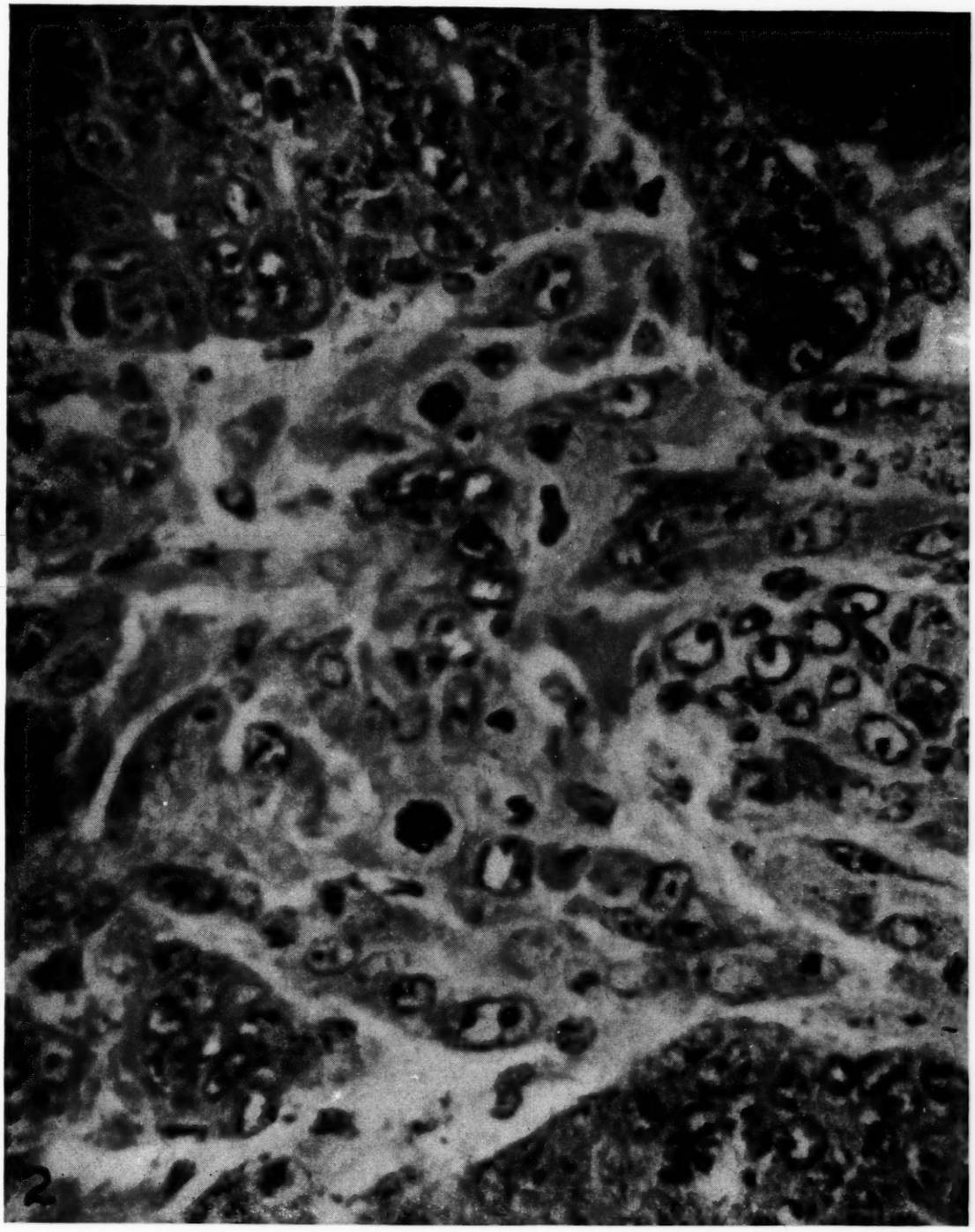


FIG. 2.—Mammary carcinoma transplant. Dark masses of cells in upper and lower portions are mammary carcinoma cells. Middle lighter portion is stromal tissue which is undergoing marked mitotic activity. Mag. × 960.

general classification of the new tumors obtained. In Table II, where the diagnosis indicates spindle-cell sarcoma, the sections indicate several subdivisions in type. The same is true for the round-cell sarcomas with the exception of the lymphosarcoma which is a specific type. This tumor appeared to develop in the right inguinal lymph node which was enveloped by the transplant.

The new tumors tended to be more malignant than the host tissue on the basis of rate of growth of transplants and the time required to kill the mouse bearing the tumor. Transplants of all these new tumors grew

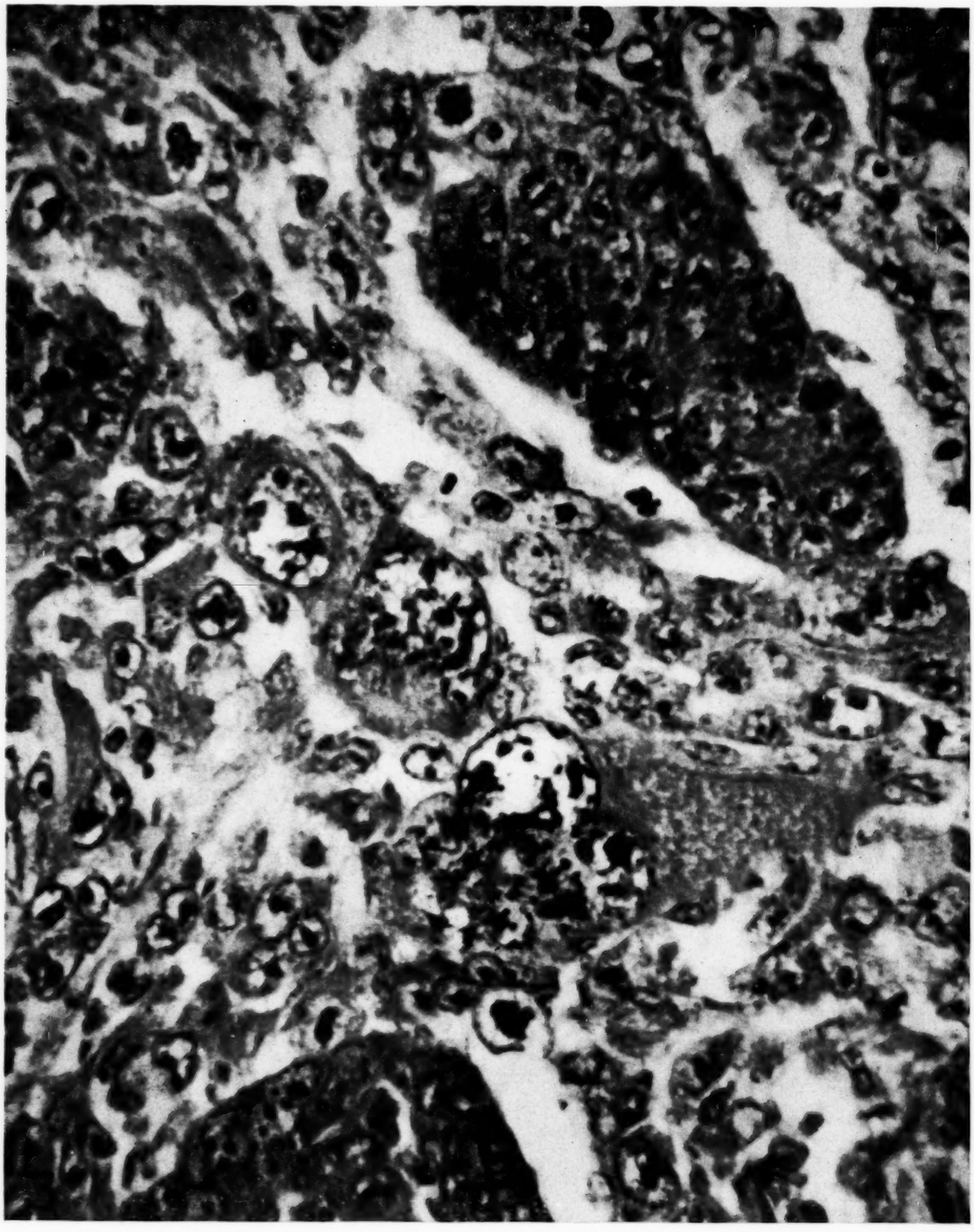


FIG. 3.—Mammary carcinoma transplant. Dark masses of cells in upper right and lower left corners are mammary carcinoma cells. The remainder of the picture consists of stromal tissue containing giant cells, and unusual mitotic activity. Mag. × 960.

readily and no further change in histology was noted even after as much as 45 transplant-generations (Fig. 5).

As Table I shows, the induction of malignancy in normal cells of the mouse by implants of egg-cultivated tumor could be demonstrated quite regularly. However, as the data given in Fig. 1 discloses, there were periods when the inducing effect was much intensified and other times when it became relatively mild.

DISCUSSION

The results show that induction of malignancy in normal contiguous cells was a frequent occurrence when

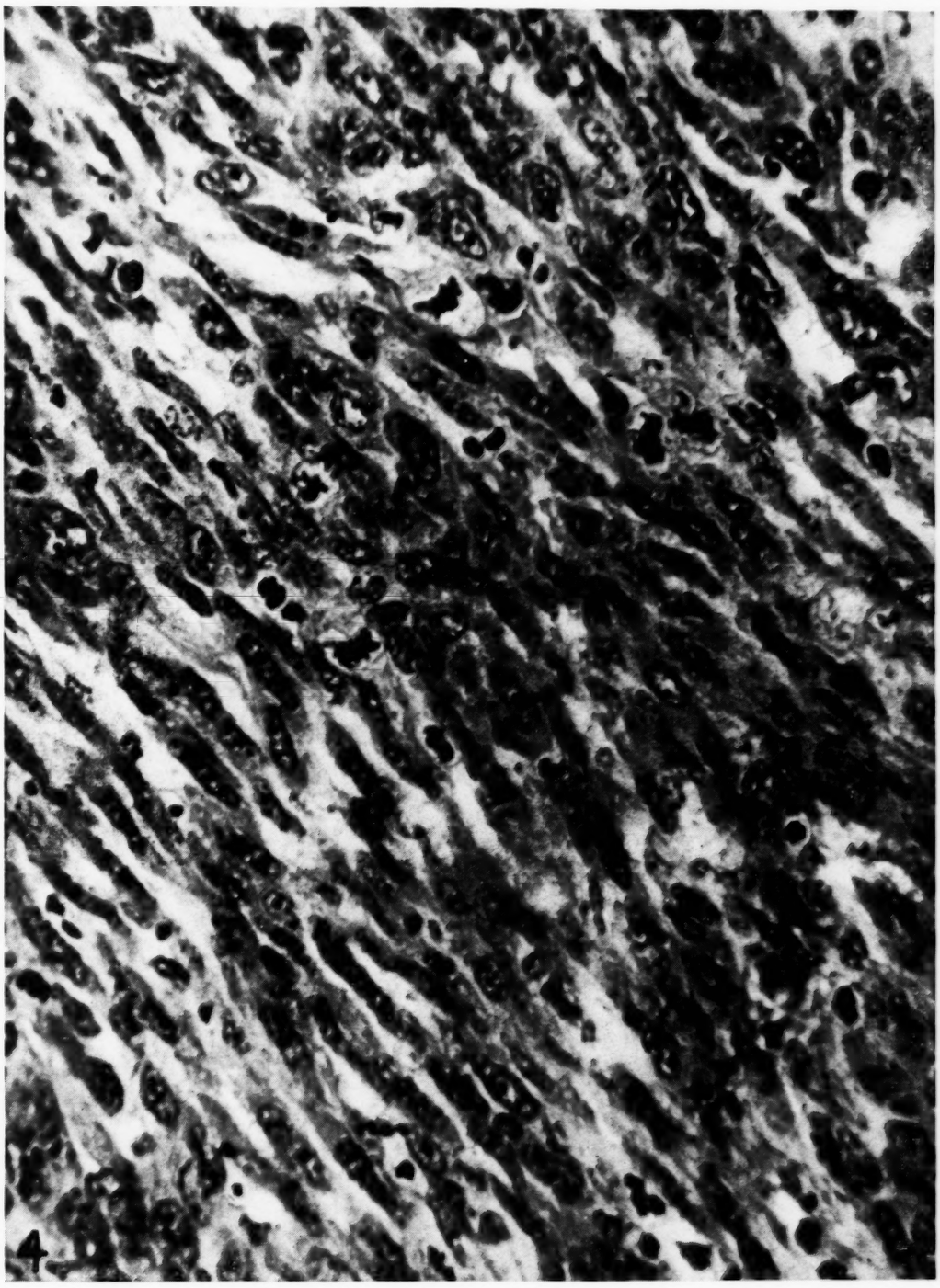
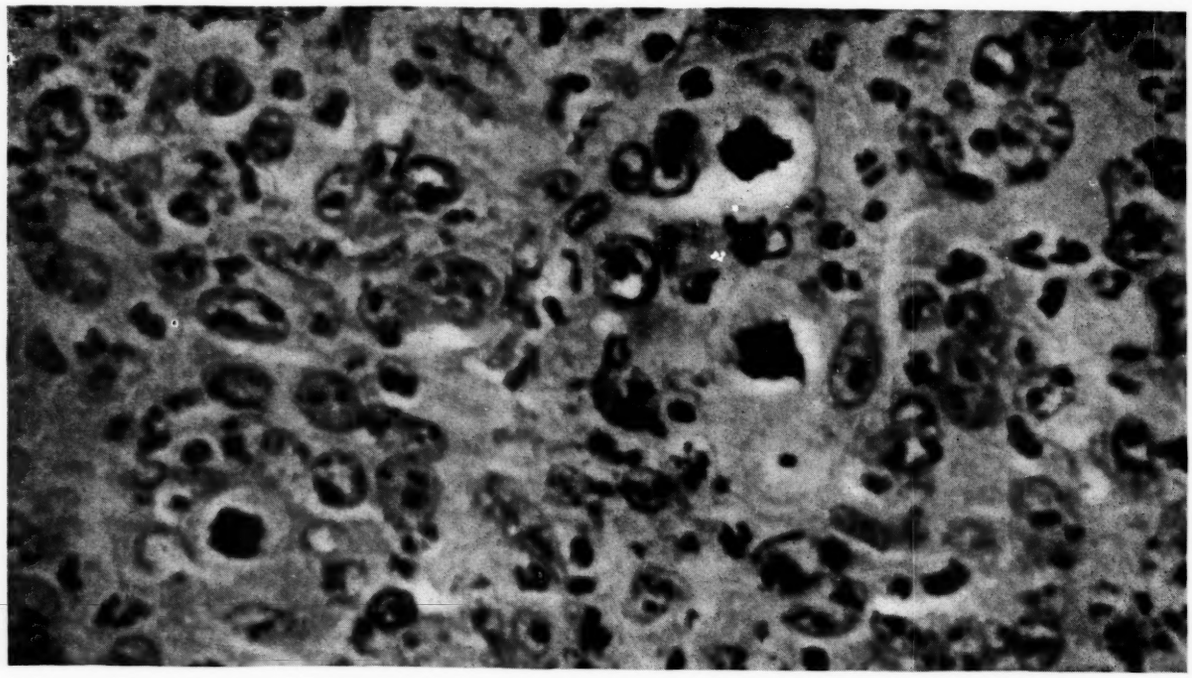


FIG. 4.—Spindle-cell sarcoma transplant originating in stroma of a mammary carcinoma transplant. Mag. × 960.

egg-cultured mouse mammary carcinoma was implanted back into mice. Sarcomatous tissue could be observed in 38 per cent of the first mouse transplant-generations with an increasing tendency toward stromal malignancy in succeeding transplants.

The egg-grown tumor tissue does not come into contact with normal cells of mouse origin so that under these conditions the mouse mammary carcinoma retains indefinitely the histology it possessed at the time the continuous egg culture was initiated. Accordingly a relatively pure line of mammary carcinoma is available for repeated implants into mice.

Previous reports of mouse mammary carcinomas which showed the development of sarcomatous tissue



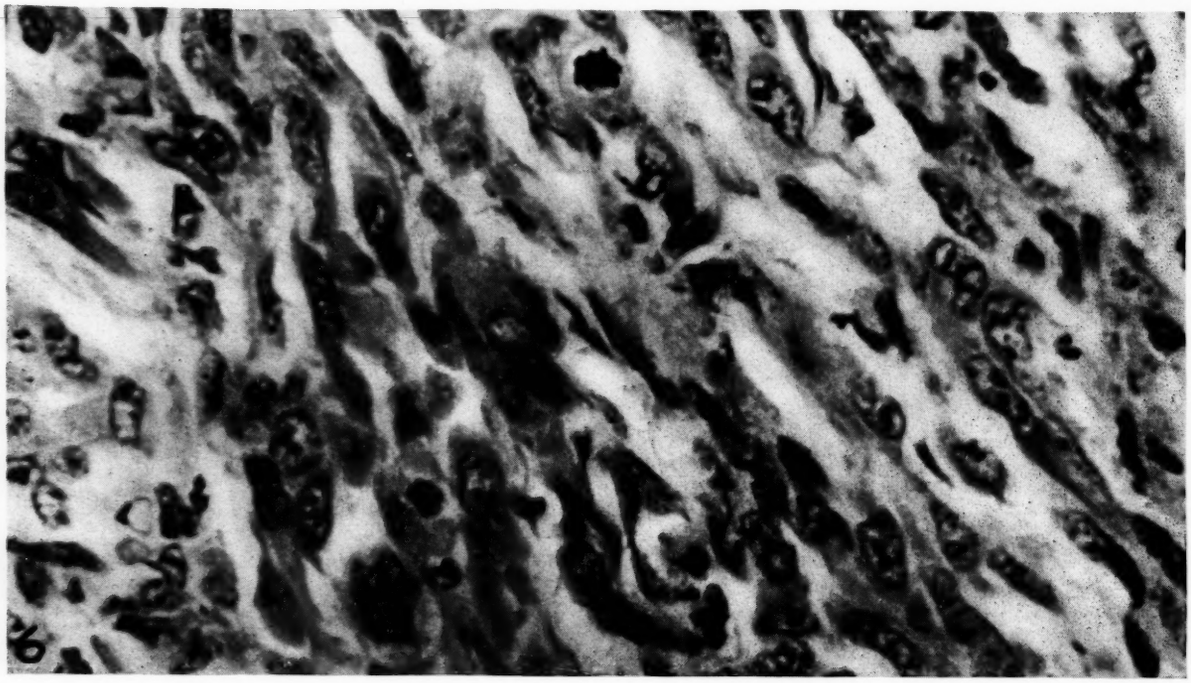
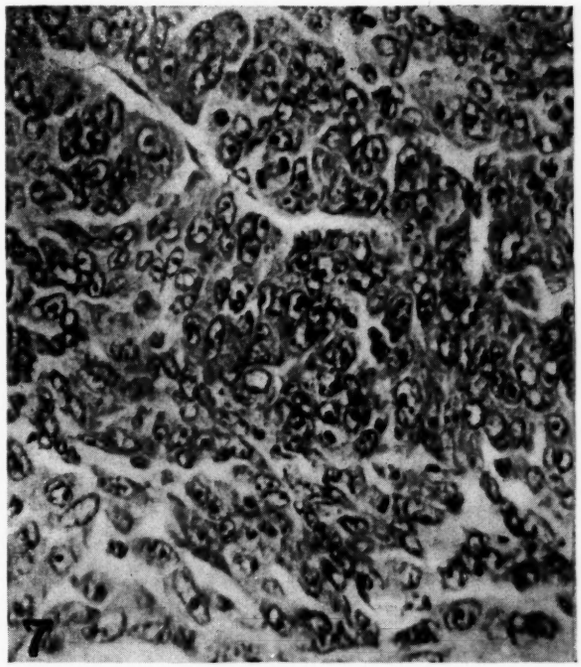
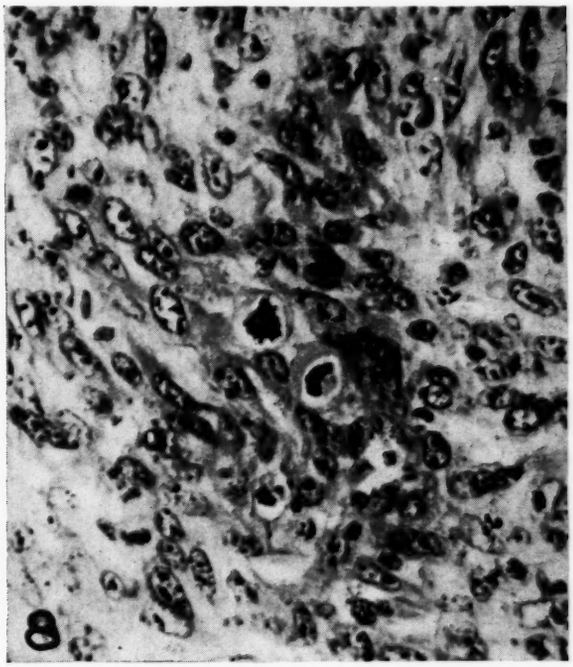


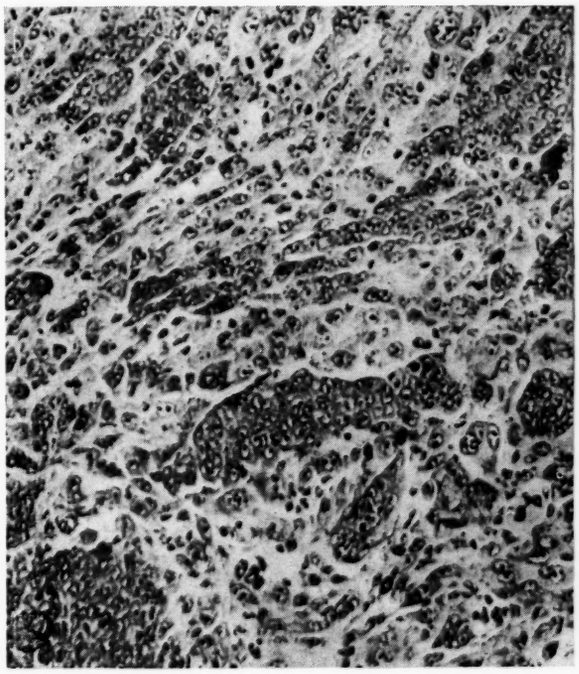
FIG. 5.—Unclassified sarcoma transplant originating in stroma of a mammary carcinoma transplant. Mag. × 960.

FIG. 6.—Spindle-cell sarcoma transplant originating in stroma of a mammary carcinoma transplant. Mag. × 960.

after a varying number of transplant-generations were based on a study of a few individual tumors. Each carcinoma behaving in this manner could be the subject of only one experiment in this regard, since, when transformation to a sarcoma was complete, the original tumor was lost. The objection can be made that the sarcomatous tissue was present from the beginning and became evident as a result of changes in the relative growth rates of the two types of tumor tissue. Such an explanation cannot be used to explain the present results. The egg-grown tumor when implanted in mice was associated with the development of sarcomas of different







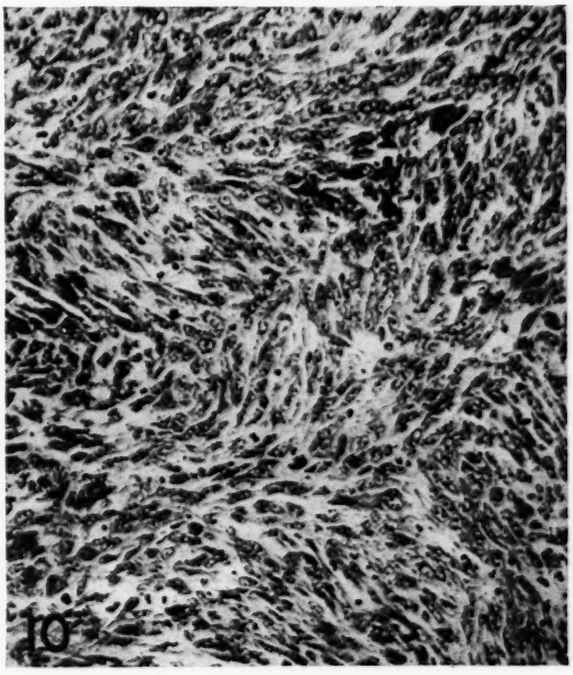


FIG. 7.—Mammary carcinoma transplant. Mag. × 400.

FIG. 8.—Unclassified sarcoma transplant originating in stroma of a mammary carcinoma transplant. Mag. × 400.

FIG. 9.—Mixed mammary carcinoma and unclassified sarcoma. Mag. × 200.

FIG. 10.—Spindle-cell sarcoma transplant originating in stroma of a mammary carcinoma transplant. Mag. × 200.

cell origins. It is unlikely that several types of sarcomatous tissue could have been present in the original mammary tumor and escaped observation during a period of nearly 5 years in which time hundreds of sections of this tumor were given careful study. Further, in some instances, no sarcomatous tissue was observed in the mammary tumor after several transplant-generations in mice.

It can be objected that these malignant growths are the original mammary carcinoma which has undergone

superficial changes in structure. There are several reasons for discarding this possibility. These can be summarized as follows:

1. The new tumors have a higher growth rate. In some instances 11 to 12 day implants are as large as 15 or 17 day implants of the donor tumor.

2. The histological appearances of the new tumors are of many types. To account for this it would have to be postulated that the mammary carcinoma was capable of assuming many different forms. Also, the histology of each tumor remains constant through numerous generations of transplants which is contrary to what happens in the instance of superficial structure changes.

3. The tumors are observed to originate in the stroma in most instances.

4. The new tumor tissue quite commonly contains giant cells, nuclear debris and mitotic aberrations not observed in the host tissue.

5. Centrifugation of mixed donor and new tumor separates the two types into a lower and upper layer respectively.

The new tumors were at least as malignant as the host tissue. Transplants in mice were 100 per cent effective and the tumor could be maintained for an indefinite period. One tumor (Fig. 5) has been carried by serial transplants in mice for more than 2 years. Many of these tumors also were cultivated successfully in eggs by the yolk sac method. Passage through eggs did not change their histology.

The speed with which this process of tumor induction in the stroma occurs is comparable to the time required for cell-free extracts to induce malignancy in normal cells (11). Implants were allowed to grow in the mouse for about 15 days and this period was sufficient for the establishment of numerous areas of sarcomatous tissue in the first transplant-generation of the source egg material.

On the basis of a large number of experiments with cell-free extracts of materials from tumor-bearing eggs, it was suspected that there was a periodicity in the activity of the tumor agent. This idea has received support in the present investigation. However, it will be necessary to investigate this aspect of the problem much more extensively before coming to a definite conclusion.

The simplest explanation for the results obtained would seem to be the assumption that normal cells of the connective tissue have been infected by the tumor agent as a result of contiguity with cancer cells. Apparently the process is essentially the same as the production of tumors by cell-free extracts of egg-grown tumor tissue. This is especially likely in view of the close similarity between sarcomas produced by implants of fresh egg-grown tumor tissue and those induced by lyophilized extracts of materials from tumor-bearing eggs. In both instances, giant cells, unusual mitotic aberrations and nuclear debris were common (11).

The probability that the tumor agent concerned in these experiments is a virus has been considered elsewhere (7). The ability of the agent as obtained from living carcinoma cells to infect and induce malignancy in connective tissue cells may appear to contradict this thesis. The Rous tumor virus, for example, appears to infect only a definite type of tissue (5). However, many viruses do not show this degree of specificity. Also viruses in general are exceedingly prone to transformations which affect their behavior in this regard. It is possible in these experiments that the tumor virus does undergo some change before being capable of infecting the cells of stroma. In many experiments there was no sign of sarcomatous transformations in the connective tissue after many mouse transplant-generations. In those instances, the tumor agent appeared to be incapable of inducing malignancy in the cells of the stroma.

One noteworthy aspect of the data is contained in the diversity of the tumors obtained. Figs. 4, 5, 6, 8 and 10 show various spindle-cell sarcomas, and two unclassified sarcomas which were carried through numerous generations of mouse transplants without showing any deviation from their initial histology. If the tumor virus from one type of carcinoma is capable of producing such definite types of tumors, then the virus concept of cancer causation may not require the postulation of as many different types of tumor agents as has been supposed.

SUMMARY

1. A mammary carcinoma from a dba mouse (dba mammary carcinoma 1) has been cultivated continuously in eggs for 2 years and 11 months, or 90 transplant-generations. During that period, there has been no observable change in the histology of the tumor tissue. The stroma of these egg-grown tumors is chick tissue.

2. Implants of the egg-grown mammary carcinoma into mice resulted in mixed carcinoma-sarcomas in 38 per cent of the first mouse transplants. The new tumor-type usually arose as isolated patches in the stroma which in subsequent generations coalesced, dividing and encircling the original carcinoma. The proportion of the transplants containing sarcomatous tissue increased with further transplant-generations in mice. A total of 186 experiments involving the use of 2,440 mice were used in the study.

3. Sarcomas produced in this manner were of different cell origins, varying growth rates and cytological characteristics. Many contained giant cells, nuclear debris, and unusual mitotic aberrations.

4. When egg-grown tumor is implanted back into the mouse, the stroma of the resulting tumor is composed of mouse tissue. It is concluded that the induction of malignancy in normal cells of the stroma is due to contiguity with the cancer cells of the implant, and consequent infection with the tumor agent or virus.

The process is considered to be essentially the same as that involved in the production of malignant tumors by injection into the mouse of cell-free extracts of materials from mouse tumor-bearing eggs.

REFERENCES

1. Bryan, W. R., Kahler, H., and Riley, V. T. Attempts to Demonstrate a Virus-Like Principle in Mammalian Tumors by the Yolk Injection Technique. The American Association for the Advancement of Science, Conference on Cancer. 1945, pp. 40-53.
2. Duran-Reynals, F. The Age Factor in Adaptability of a Sarcoma Virus to Other Animal Species. Science, **103**:748-749. 1946.
3. Heilman, F. R. On Yolk Sac Cultivation and Virus Induction of Malignant Tumors. The American Association for the Advancement of Science, Conference on Cancer. 1945, pp. 54-55.
4. Ludford, R. J., and Barlow, H. Sarcomatous Transformation of the Stroma of Mammary Carcinomas that Stimulated Fibroblastic Growth *in Vitro*. Cancer Research, **5**:257-264. 1945.
5. Rous, P. Viruses and Tumors. In Virus Diseases. Ithaca: Cornell University Press. 1943, p. 147.
6. Taylor, A. The Successful Production of a Mammalian Tumor with a Virus-Like Principle. Science, **97**:123. 1943.
7. Taylor, A. The Virus Concept in Cancer Research. University of Texas Publication No. 4507. 1945, pp. 43-46.
8. Taylor, A., Hungate, R. E., and Taylor, D. R. Yolk Sac Cultivation of Tumors. Cancer Research, **3**:537-541. 1943.
9. Taylor, A., and Kynette, A. Evidence for a Cancer Virus Using Intraocular Implants of Mouse Tumor in Rats. University of Texas Publication No. 4507. 1945, pp. 33-42.
10. Taylor, A., and Kynette, A. The Virus Production of Mammalian Tumors. American Association for the Advancement of Science Research Conference on Cancer. Lancaster, Pennsylvania: The Science Press Printing Company. 1944, p. 24.
11. Taylor, A., Kynette, A., and Hungate, R. E. Demonstration of a Virus-Like Tumor Agent by Filtration and Desiccation of Materials from Eggs Bearing Mouse Mammary Carcinoma. University of Texas Publication No. 4507. 1945, pp. 13-31.
12. Taylor, A., Thacker, J., and Pennington, D. Growth of Cancer Tissue in the Yolk Sac of the Chick Embryo. Science, **96**:342-343. 1942.
13. Twombly, G. H., and Meisel, D. The Growth of Mammalian Tumors in Fertile Eggs. Is a Filterable Cancer Virus Produced? Cancer Research, **6**:82-91. 1946.

Production of Tumors in Rats by 2-Aminofluorene and 2-Acetylaminofluorene

Failure of Liver Extract and of Dietary Protein Level to Influence Liver Tumor Production

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(Received for publication August 22, 1946)

The carcinogenic property of 2-acetylaminofluorene was first described by Wilson and his associates (9) in 1941. Of 39 rats that received the compound in the food at a level of 0.031 to 0.125 per cent for 95 or more days, 19 developed malignant tumors, and 8 animals had multiple tumors. These included 10 bladder carcinomas; 8 epidermoid carcinomas of the side of the face; 3 mammary carcinomas; 3 hepatic carcinomas; 1 carcinoma arising in each of the following sites: ureter, kidney pelvis, colon, pancreas, and lung; and 1 rhabdomyosarcoma of the thigh. The rats were derived from the Slonaker strain, and the majority were female. One-half gram of the compound was injected subcutaneously into 5 male rats, and no tumors had developed 14 months later.

In 1944 Bielschowsky (2), using rats derived from the Wistar strain, and feeding at the level of 4 mgm. of the carcinogen per day, found malignant tumors in 93 of 104 animals. Some animals had multiple tumors. Fifty-five rats had liver carcinoma, 27 had mammary carcinoma, 16 had carcinoma of the acoustic duct, 5 had carcinoma of the intestine, 1 had carcinoma of the skin, and 1 had a tumor of the uterus. In addition, 4 animals developed leukemia, and 11 had pulmonary adenomas. In male rats liver tumors were most numerous, and mammary carcinoma was relatively uncommon, but in female rats mammary carcinoma was twice as common as liver carcinoma.

Bielschowsky also found the compound to be innocuous when injected subcutaneously, and surmising that the subcutaneous tissues could not split off the acetyl group, applied a 4 per cent solution of 2-aminofluorene in acetone thrice weekly to the skin of 5 male rats for a period of 210 days. After 280 days, liver tumors were found in all 5 rats, and 1 rat also had an acoustic duct tumor.

By simultaneous feeding of 2-acetylaminofluorene and allylthiourea, Bielschowsky (3) produced benign and malignant thyroid tumors, but by successive feeding of these compounds (4) thyroid tumors were not produced. Carcinomas of the small intestine, liver, mammary gland, and acoustic duct developed in some of the rats in both experiments.

Armstrong and Bonser (1) administered 0.2 cc. of a 1.5 per cent suspension of 2-acetylaminofluorene in olive oil by gavage thrice weekly to CBA mice, and of 10 that survived 32 to 65 weeks of treatment, 8 developed tumors. Five had bladder tumors (4 were malignant); 5 had liver tumors (1 was malignant); and 2 had uterine tumors.

Lopez (7) reported development of a glioblastoma of the cerebrum in 1 of 12 rats that had been fed a diet containing 0.05 per cent of 2-acetylaminofluorene. At the time his article was written, 7 rats were still living and 4 had developed tumors. In addition to the brain tumor, there were 2 liver tumors, 2 mammary tumors, and 3 acoustic duct tumors.

Heiman and Meisel (6) administered 2-acetylaminofluorene to 59 Wistar rats by introducing a needle into the pharynx and expelling 1 cc. of peanut oil containing 10 mgm. of the carcinogen. At first, treatment was given every other day, but later the dose was doubled and given every third day. Twenty-two rats developed nodular swellings in one or both submaxillary regions. Subsequently, enlarged submaxillary glands and oil-containing cysts were found, the latter having resulted from perforation of the pharynx. Only 12 animals developed tumors. These included 2 adenocarcinomas and 3 adenomas of the submaxillary glands (the latter appearing as isolated areas of hyperplastic ducts and acini), 2 parathyroid adenomas, 2 mammary carcinomas, 1 thyroid adenoma, 1 liver adenoma, and 1 sarcoma of the neck. Twenty-four rats received aromatic amino acids over a period of 45 days and none developed tumors or hepatic cirrhosis. The authors attributed development of tumors in the vicinity of the oil cysts to local action of 2-acetylaminofluorene.

Presented at the 37th Annual Meeting of the American Association for Cancer Research at Atlantic City, New Jersey, March 11, 1946.

In view of the high incidence of liver tumors in Bielschowsky's rats, it was proposed to ascertain whether or not some of the dietary factors that are known to influence the carcinogenic action of *p*-dimethylaminoazobenzene would have the same effect upon carcinogenesis by 2-acetylaminofluorene and 2-aminofluorene. Since the available amount of these 2 compounds was small, only a few experiments could be carried out.

TABLE I: COMPOSITION OF DIETS

	Basal A	Basal B	Basal C
Labco vitamin-free casein	20.0	13.0	10.0
Primex	5.0	7.0	5.0
McCollums salt mixture no. 185 (modified)	4.0	4.0	4.0
Carotene	0.001	0.001	0.001
Vitamin D concentrate in cottonseed oil (400,000 U./gm.)	0.0005	0.0005	0.0005
Thiamin	0.0005	0.0003	0.00025
Riboflavin	0.0008	0.0002	0.00015
Pyridoxine	0.0003	0.0003	0.0002
Nicotinic Acid	0.001	0.002	0.001
Distilled Natural Tocopherols	0.001	0.001	0.001
Calcium Pantothenate	0.00056	0.0007	0.00056
Choline Chloride	0.10	0.10	0.0
Starch	70.9	75.9	81.0

	gm.		gm.
Diet 1		Diet 23	
Basal A	999.6	Basal A	970
2-Acetylaminofluorene	0.4	3% Solution of *p*-dimethylaminoazobenzene in cottonseed oil	30
	1000.0		1000
Diet 2		Diet 25	
Basal B	999.6	Basal C	980
2-Acetylaminofluorene	0.4	3% Solution of *p*-dimethylaminoazobenzene in cottonseed oil	20
	1000.0		1000
Diet 3		Diet 26	
Basal B	969.6	Basal C	950
Liver Extract, Lilly	30.0	Liver Extract, Lilly	30
2-Acetylaminofluorene	0.4	3% Solution of *p*-dimethylaminoazobenzene in cottonseed oil	20
	1000.0		1000

METHODS

Our rats were obtained from a local breeder and were descended from the Wistar strain. The carcinogens were obtained from Eastman Kodak Company. The 2-acetylaminofluorene was incorporated in the diet at a level of 0.04 per cent and the rats were fed *ad libitum*. The 2-aminofluorene was dissolved in benzene in a concentration of 4 per cent and was painted on the abdominal skin for 147 days (from October 20, 1944 until March 16, 1945). Applications were made twice weekly for 3 months and 5 times weekly for 2 months, with a total of 62 treatments.

The rats that received the acetyl derivative were divided into three groups, each of which was placed on a different synthetic diet (diets 1, 2, and 3). These three diets were chosen because they were very similar to diets that we (5) have found to influence profoundly *p*-dimethylaminoazobenzene carcinogenesis (see Fig. 1, curves 23, 25, and 26). The composition of these diets is given in Table I. Diet 1 is comparable to diet 23, but contains 3 per cent less fat. Diets 2 and 3 are comparable to diets 25 and 26, respectively, but differ in containing 3 per cent more casein and greater amounts of some of the members of the vitamin B group. These differences were introduced with the idea that use of basal diet C with 2-acetylaminofluorene might be accompanied by a high mortality rate, and that slight increase in certain factors, which experience had shown would have little effect upon *p*-dimethylaminoazobenzene carcinogenesis, might also have little effect upon 2-acetylaminofluorene carcinogenesis, but appreciable capacity to diminish the mortality rate. It now appears probable that this precaution was unnecessary.

The rats that were painted with the solution of 2-aminofluorene were divided into two groups. One group was fed with basal diet B (diet 4), and the other was fed with our regular colony diet (diet 5), an excellent ration that promotes rapid growth.

After 4 months of treatment, the rats were examined weekly for tumors. The presence of liver tumors was determined by palpation of the abdomen. This procedure was not entirely satisfactory, since the fluorene derivatives usually caused considerable enlargement of the liver before tumors developed. Even when tumors appeared, they often protruded only slightly above the surface of the surrounding tissue and could not be palpated readily. As soon as it seemed certain that a liver tumor was present, the rat was killed and its viscera

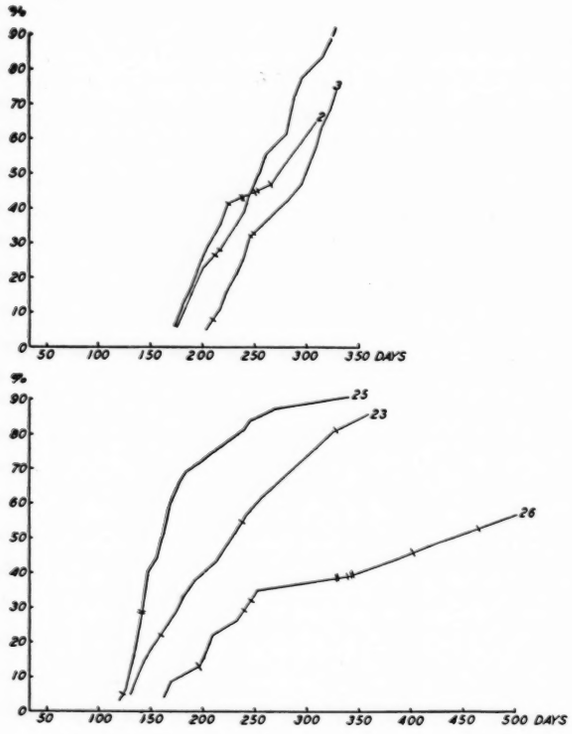


FIG. 1.—For explanation, see the first paragraph under Results.

were examined. A few animals were killed because of mammary or acoustic duct tumors that had reached a size or condition deleterious to the animal's health, and in some of these liver tumors were found. Consequently, in some animals tumors were found soon after they had developed, and in others tumors had been present for several weeks before they were discovered.

RESULTS

It will be seen by reference to Fig 1, which shows percentage incidence of liver tumors plotted against time, that variation in casein and riboflavin content of the diet had no effect upon production of liver tumors by 2-acetylaminofluorene (curves 1 and 2). Liver extract appeared to retard tumor development slightly (curve 3), but the effect is of doubtful significance. For all diets in Fig. 1, death of a tumor-free animal or of 1 that had other than a liver tumor is indicated by a short line perpendicular to the curve. For comparison, the effects of liver extract (curve 26), and of increased amounts of riboflavin and casein (curve 23), as contrasted with a diet low in riboflavin and casein (curve 25), upon *p*-dimethylaminoazobenzene carcinogenesis are shown in the same figure. Diet 26 greatly retards development of liver tumors, and diet 23 has a similar, but weaker effect.

If female rats are excluded from the calculations as a means of minimizing the complications introduced by mammary tumors, the slope of curves 1, 2, and 3 is a little more steep and the ultimate liver tumor percentages are 100, 100, and 85, respectively, and the curve for diet 2 never crosses that of diet 1, but always runs a little ahead of it. The curves for the three diets thus have the same relative order as do those of the three corresponding *p*-dimethylaminoazobenzene diets, but the difference in the rate of tumor formation for the 2-acetylaminofluorene diets is too slight to be significant.

Although variation in the diet had no appreciable effect upon the tumor incidence, comparison of the number of animals in each group at the beginning of the experiment with the effective number (see Table II) will show that it did have a decided effect upon the mortality rate during the latent period. During this time the mortality rate on diet 1 was 28 per cent, on diet 2, 43 per cent, and on diet 3, 10 per cent. The number and location of tumors obtained are also shown in Table II.

TABLE II: TUMOR INCIDENCE ACCORDING TO SEX AND DIET

Diet number	1		2		3		4		5		Total	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
No. rats in each group	20	5	20	10	15	5	14	1	14	—	83	21
Effective no.	13	5	9	8	14	4	10	1	14	—	60	18
No. that developed tumor	13	4	9	7	12	4	8	1	11	—	53	16
Total with liver tumor	13	4	9	3	11	3	7	—	10	—	50	10
Liver tumor only	10	1	6	—	7	—	4	—	7	—	34	1
Acoustic duct tumor	2	—	—	5	2	1	4	—	3	—	11	6
Mammary tumor	—	2	—	5	—	3	—	1	—	—	—	11
Bladder tumor	—	1	1	—	1	—	—	—	—	—	2	1
Pulmonary adenoma	—	1	1	2	—	1	2	—	—	—	3	4
Other tumors*	2	1	1	—	1	—	2	—	1	—	7	1

* Including 1 adenocarcinoma of ileum, 1 epidermoid carcinoma of lung, 3 papillary epidermoid carcinomas of buccal mucosa, 1 papilloma of lip, 1 papilloma of forestomach, and 1 fibroma of flank. Two rats had a small cavernous hemangioma of liver.

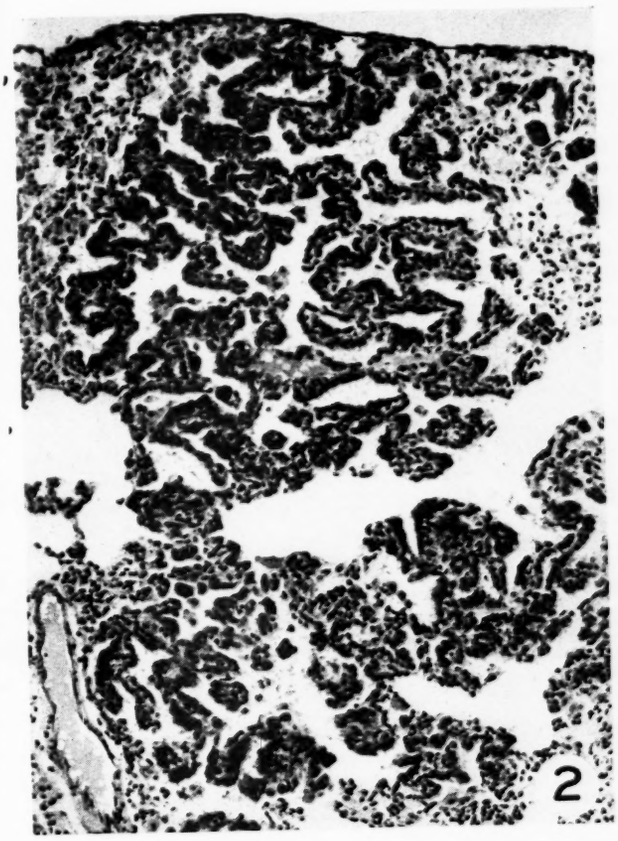
DESCRIPTION OF FIGURES 2 TO 5

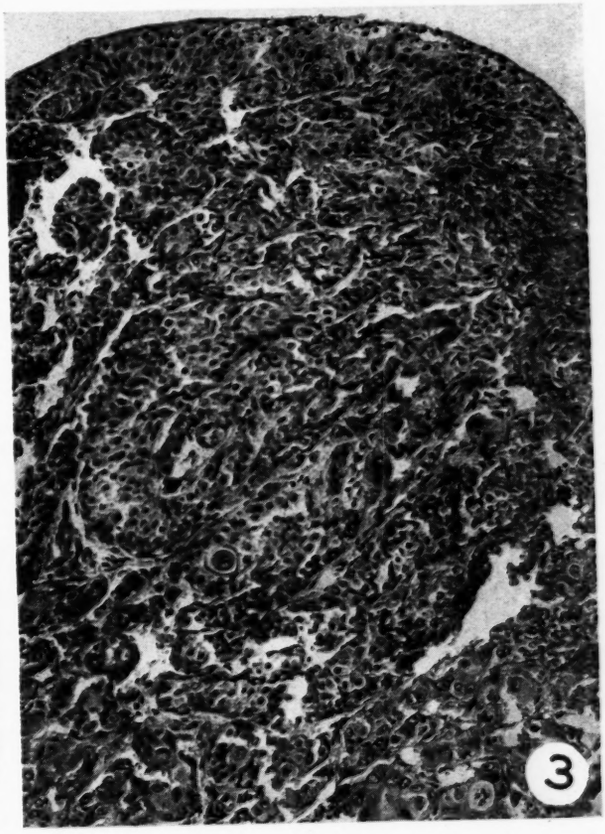
FIG. 2.—Pulmonary adenoma. Mag. × 140.

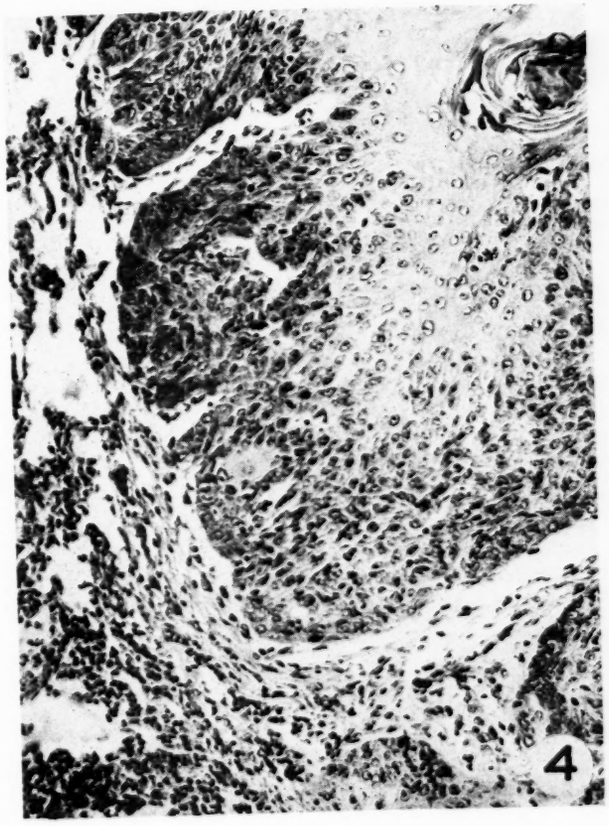
FIG. 3.—Epidermoid carcinoma of bladder. Mag. × 140.

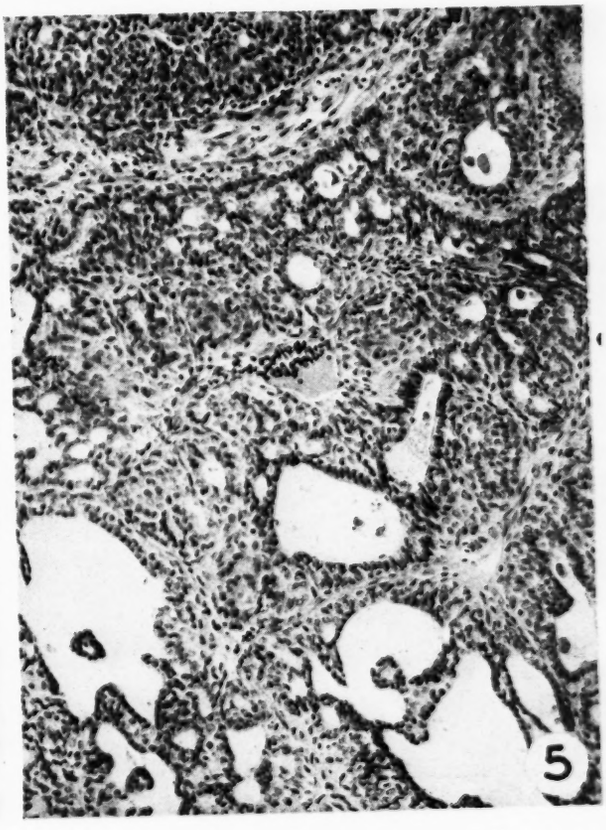
FIG. 4.—Epidermoid carcinoma of acoustic duct. Mag. × 140.

FIG. 5.—Mammary carcinoma. Mag. × 140.

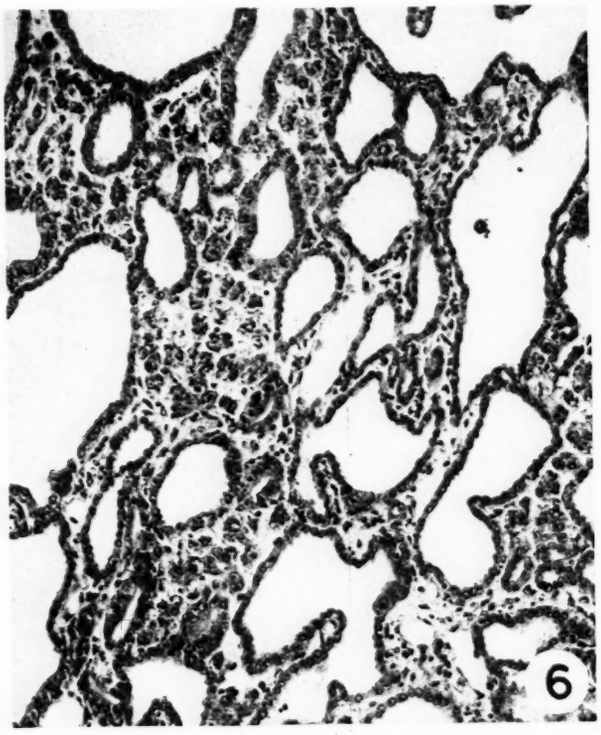


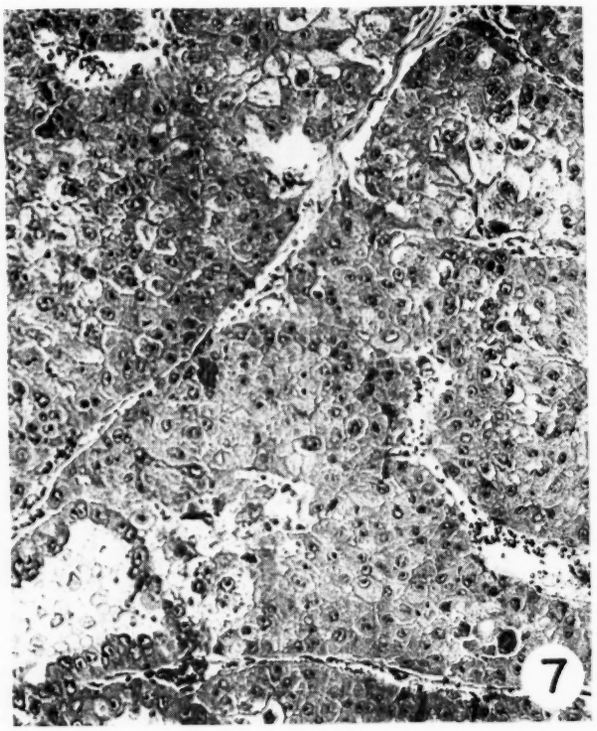


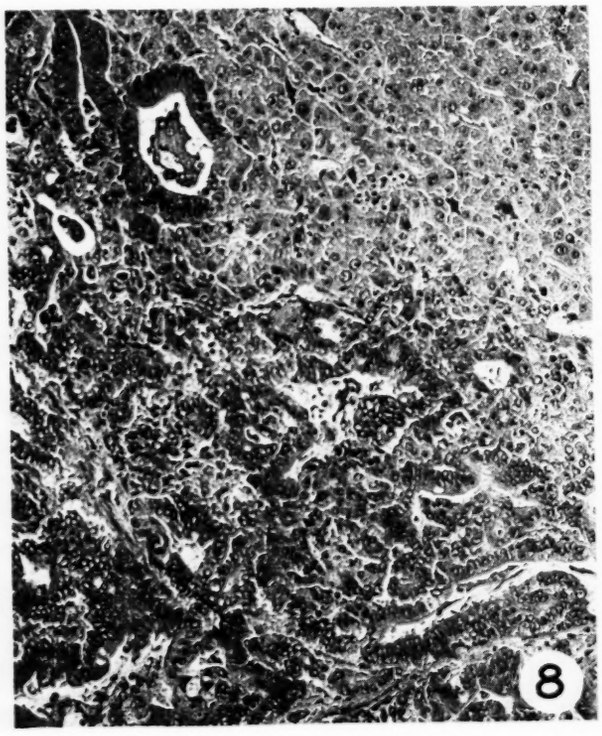


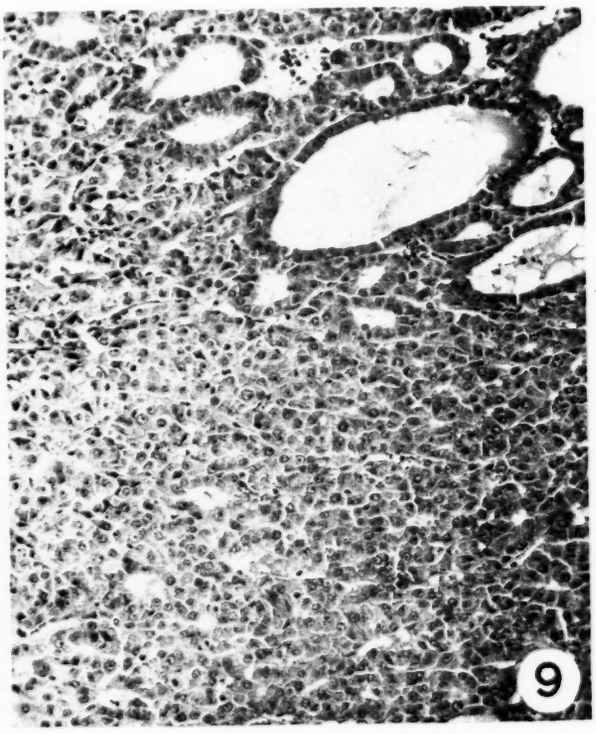


FIGS. 2-5









FIGS. 6-9

The number of rats that developed liver tumors following painting with 2-aminofluorene was too small to justify much discussion of the effect of diet, but certain facts are of interest. Four rats on diet 4 died of pneumonia 125 to 137 days after treatment was begun, and 2 rats on diet 5 died of pneumonia 157 and 171 days after painting was begun. It was thought that the stock diet (no. 5) might offer protection against tumor development, yet the first liver tumor was found at 137 days in a rat getting this diet, and 2 more rats in this group had developed liver tumors by the time the first liver tumor was found in the other group (diet 4). However, when liver tumors began to appear on diet 4 they did so rapidly (the latent period ranged from 242 to 322 days), and the slope of the curve of tumor incidence made an angle of 60° with the abscissa. The latent period on diet 5 ranged from 137 to 405 days, and the slope of the curve of tumor incidence made an angle of 30° with the abscissa. Hence, there is no clear-cut evidence that diet had any effect upon development of liver tumors.

The acoustic duct tumors were all epidermoid carcinoma. The majority were of low grade malignancy and were sharply circumscribed. They consisted of large papillary masses of squamous epithelial cells with a delicate fibrous tissue core. The surface of the masses was covered with a thick layer of keratinized cells. Fig. 4 presents an example. Two of the tumors showed much anaplasia and were highly invasive; in several of the better differentiated tumors, there was beginning invasion of the surrounding tissues. Many of these tumors were infected by the time they were removed for section. They had a sour odor and were filled with flaky gray material. Two rats had bilateral tumors.

One mammary tumor was of intracystic papillary type and appeared benign. The other mammary tumors were, with one exception, adenocarcinomas of various grades of malignancy (see Fig. 5). The exception was an epidermoid carcinoma. One of the other carcinomas contained foci of squamous metaplasia.

The bladder tumors were all epidermoid carcinomas and were small. An example is shown by Fig. 3.

Pulmonary adenomas were found in 7 rats; they were usually multiple and small (see Fig. 2). If several blocks of lung from each rat had been sectioned routinely, the incidence of adenomas would doubtless have been found to be considerably higher. One rat had an extensive epidermoid carcinoma of the lung. No other possible primary site for this tumor was found. Moreover, the other lung was free of metastases. Six rats were found to have metastatic pulmonary tumors; the primary sites of these tumors were: acoustic duct; ileum; liver (2 cases); and mammary (2 cases, 1 of which was the epidermoid carcinoma).

Portions of exorbital lachrymal glands or parotid glands were included in sections of several acoustic duct tumors. In no case did tumors develop in these glands, but in five instances the structure of the glands was strikingly altered. Nuclei were enlarged to a variable degree, and were often greatly enlarged. The amount of chromatin was considerably increased; the nuclei were stained deeply and often had large nucleoli. Some cells contained more than one nucleus. Occasionally, nuclei were crescentic and partially enveloped large vacuoles. In view of the observations of Heiman and Meisel (6), it is unfortunate that the salivary glands were not all sectioned routinely, and it is recommended that such measures be taken in future investigations. The salivary glands of our rats were not appreciably enlarged, and we had no reason to suspect neoplasia in them. Since the conditions of our experiments were entirely different, the findings should not be construed as contradictory.

Changes in the liver warrant a more detailed discussion than has been given of the other tissues. Enlargement of the liver was observed in about a third of the rats, and was due to hypertrophy of liver cells. Hypertrophy of the liver was also observed by Wilson and his associates (9). Well developed nodular cirrhosis was seen in only 5 rats, but in 20 other animals the presence of slight cirrhosis was revealed by microscopic examination, and in the livers of an additional 35 rats there were foci in which thin fibrous tissue trabeculae radiated outward and partly surrounded a few liver lobules. The last mentioned condition is evidently an incipient cirrhosis, for extension of the process would ultimately lead to the development of true cirrhosis. In addition to increase in fibrous tissue, there was usually some increase in small bile ducts. In some places there was considerable proliferation of bile ducts with formation of clusters of ducts. Many of these bile ducts were dilated and formed cysts of various sizes. The lining cells of some cysts were greatly

DESCRIPTION OF FIGURES 6 TO 9

FIG. 6.—Liver—multiple cysts. Mag. × 140.

FIG. 7.—Liver, malignant hepatoma with broad cords of cells. Mag. × 130.

FIG. 8.—Liver, malignant tumor containing glandular and trabecular elements. Mag. × 130.

FIG. 9.—Liver, malignant hepatoma with a region of acinus formation. Mag. × 140.

attenuated, but in other cysts they were cuboidal or even low columnar in type. In places, such cysts were separated by small numbers of normal liver cells, as illustrated by Fig. 6. In many rats there was no increase in collagenous tissue in the liver. Except for hypertrophy, the changes just described are similar to those seen in the livers of rats treated with *p*-dimethylaminoazobenzene. Other differences observed were: (1) that the cysts in the livers of the rats treated with the fluorene compounds contained bile, a fact rendered obvious by fixation of the tissues, whereas the cysts in the livers of rats treated with *p*-dimethylaminoazobenzene did not; and (2) in livers of *p*-dimethylaminobenzene-treated rats a lesion which Opie (8) has named cholangiofibrosis was common, but was not encountered in rats treated with the fluorene derivatives.

In 12 rats only one liver tumor was present, and in seven instances it was a benign hepatoma. All other rats' livers contained 2 or more tumors. Some were benign hepatomas, and 3 were adenocarcinomas, but the majority were malignant hepatomas (as judged by invasion of veins or of liver tissue). In many, the cells were arranged in cords, 1 to 2 cells wide, but in some instances the cords were much broader, and, not infrequently, foci were seen in which some tumor cells were arranged about lumina. The proportion of tumors composed of cells with acidophilic cytoplasm was a little greater than in our *p*-dimethylaminoazobenzene tumors, but in general the tumors were quite similar. Examples are given in Figs. 7 to 9.

DISCUSSION

Since the site of tumor development in our rats differed so strikingly from that of Wilson and his co-workers (9), who used rats of the Slonaker strain, and agreed so well with that of Bielschowsky (2), who used rats of the same ancestry as ours, it appears that the incidence of tumors to be expected in the various tissues is determined by the strain of the rat.

The failure of the dietary modifications in these experiments to influence development of liver tumors indicates that the mechanism of tumor formation by the fluorene derivatives differs from that concerned with *p*-dimethylaminoazobenzene carcinogenesis. The ability of the former compounds to produce tumors in such a multiplicity of sites, as contrasted with the ability of the latter compound to produce liver tumors only, also suggests that the mechanism of tumor formation by the two classes of compounds is different.

There was no significant difference in tumor incidence or types of tumors developing in rats treated with 2-aminofluorene and 2-acetylaminofluorene.

SUMMARY

1. Addition of liver extract to the diet had no appreciable effect upon the production of liver tumors in rats by 2-acetylaminofluorene, and variation in the protein content of the diet had no evident effect upon the production of liver tumors by 2-aminofluorene and 2-acetylaminofluorene.

2. The site at which tumors may be induced by these two compounds is probably determined by the genetic constitution of the rat employed.

REFERENCES

1. Armstrong, Elizabeth C., and Bonser, Georgiana M. Epithelial Tumours of the Urinary Bladder in Mice Induced by 2-Acetyl-amino-fluorene. J. Path. & Bact., **56**:507-512. 1944.
2. Bielschowsky, F. Distant Tumours Produced by 2-Amino and 2-Acetyl-amino-fluorene. Brit. J. Exper. Path., **25**:1-4. 1944.
3. Bielschowsky, F. Tumours of the Thyroid Produced by 2-Acetyl-amino-fluorene and Allyl-thiourea. Brit. J. Exper. Path., **25**:90-95. 1944.
4. Bielschowsky, F. Experimental Nodular Goitre. Brit. J. Exper. Path., **26**:270-275. 1945.
5. Harris, P. N., Krahl, M. E., and Clowes, G. H. A. *p*-Dimethylaminoazobenzene Carcinogenesis with Purified Diets Varying in Content of Cysteine, Cystine, Liver Extract, Protein, Riboflavin and Other Factors. To be published.
6. Heiman, J., and Meisel, D. Tumors of the Salivary and Parathyroid Glands in Rats Fed with 2-Acetyl-amino-fluorene. Cancer Research, **6**:617-619. 1946.
7. Lopez, E. V. Glioma in a Rat Fed with 2-Acetyl-amino-fluorene. Nature, London, **156**:296-297. 1945.
8. Opie, E. L. The Pathogenesis of Tumors of the Liver Produced by Butter Yellow. J. Exper. Med., **80**:231-246. 1944.
9. Wilson, R. H., De Eds, F., and Cox, A. J. The Toxicity and Carcinogenic Activity of 2-Acetaminofluorene. Cancer Research, **1**:595-608. 1941.

The Metabolism of *N,N*-Dimethyl-*p*-Aminoazobenzene and Related Compounds

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(Received for publication November 14, 1946)

Studies on the metabolism of azo compounds related to *p*-aminoazobenzene in the rat so far have indicated that: (a) Fission of the azo linkage occurs (4, 12); (b) Dealkylation of *N*-methyl derivatives (10) and the *N*-ethyl derivative (5) occurs, at least to some extent, prior to reduction fission; (c) Partial reduction to hydrazo compounds which undergo a benzidine rearrangement in acid solution may also occur (2, 3).

The results obtained in this laboratory bearing on this problem are reported below.

METHODS

Three per cent solutions of the compounds in cottonseed oil were mixed with the diet of brown rice and carrot. This diet has been shown to favor the production of liver cancer (14) in the rat.

N,N-dimethyl-*p*-aminoazobenzene (DMB), *N*-methyl-*p*-aminoazobenzene (MMB), and *p*-aminoazobenzene (AB) were determined by chromatographic analysis and absorption characteristics in acid solution essentially as outlined by the Wisconsin group (9). *N,N*-diethyl-*p*-aminoazobenzene (DEB) was absorbed less firmly on aluminum oxide[1] than was DMB. *N,N*-diethanol-*p*-aminoazobenzene (DE-ol-B) was absorbed much more strongly and did not elute with benzene but was eluted with a 2:1 benzene-methanol mixture.

The absorption maxima of these azo compounds in 7N HCl were determined in a Beckman spectrophotometer and are as follows:

AB	500 mμ.
MMB	508 mμ.
DMB	518 mμ.
DEB	520 mμ.
De-ol-B	528 mμ.

The absorption spectra were measured between 240 and 560 mμ. Other absorption maxima were found at 320 and 270 mμ. but the absorption coefficients were much lower than at the 500 mμ. bands.

[1] Merck-Brockmann aluminum oxide partially inactivated by mixing with methanol and drying at 90° C. (9).

In the tissue slice experiments 200 mgm. (wet weight) of tissue were used. The medium consisted of 3 ml. of Krebs-Ringer-phosphate, 0.4 ml. of 2 per cent glucose, and 0.1 ml. of 95 per cent ethanol containing 50 μgm. of the azo compound. The tissues were incubated for 90 minutes at 37.5° C. in test tubes and shaken in a Warburg bath. At the end of the incubation period the contents of the tubes were ground in a glass homogenizer. The extraction procedure used was the same as for tissue samples (9).

In the brown rice diet experiments a 3 per cent solution of the compound was added to the diet to make the concentration of the dye 0.06 per cent, the diet mixed, an aliquot weighed out, and the material ground and extracted in the same manner as the tissues.

RESULTS

Ten rats were fed the brown rice and carrot diet containing 0.06 per cent *N,N*-dimethyl-*p*-aminoazobenzene (DMB). The rats were killed and the tissues were analyzed for DMB, MMB, and AB. The results obtained are in agreement with those of the Wisconsin group with the exception of the distribution of the 3 dyes in the stomach contents. The results of typical experiments are shown in Table I.

The finding that a considerable amount of the DMB had been demethylated to MMB and AB in the stomach contents led us to investigate the stability of DMB in the brown rice diet. The results are shown in Table II. It can be seen that demethylation of DMB in the diet fed was responsible for the unexpected finding of large amounts of MMB and AB in the stomach contents. Only a very small amount of DMB was found to be demethylated in 4 weeks in cottonseed oil, whereas demethylation occurred immediately on mixing with the brown rice diet. As is shown in Table II if the brown rice diet was heated at 90° C. for 5 days before DMB was mixed with it, the demethylation did not occur. During the heating period the weight of the brown rice decreased between 10 to 14 per cent. The mechanism of removal of the methyl groups is not known.

TABLE I: DISTRIBUTION OF DMB, MMB AND AB IN TISSUES OF RATS FED A BROWN RICE DIET CONTAINING 0.06% DMB FOR 30 TO 40 DAYS

	Liver γ/organ			Spleen γ/organ			Blood cells γ/ml.			Stomach contents Total γ			Serum γ/ml.		
Rat no.	DMB	MMB	AB	DMB	MMB	AB	DMB	MMB	AB	DMB	MMB	AB	DMB	MMB	AB
1	1.0	1.0	5.6	..	..	..	0	0	22.0	...	...	..	0	0	0
2	1.8	2.2	10.6	..	..	..	0	0.5	28.0	85.0	23.5	5.0	0	0	0.5
3	1.2	2.6	5.8	0	0	5.6	..	..	...	23.4	3.8	1.0	..	..	..

DMB = *N,N*-dimethyl-*p*-aminoazobenzene
MMB = *N*-methyl-*p*-aminoazobenzene
AB = *p*-aminoazobenzene

When DEB or DE-ol-B in cottonseed oil was mixed with the diet no detectable de-ethylation or de-ethanolation occurred. As shown in our previous report (5), the stomach contents of rats fed DEB contained only that dye in detectable amounts.

TABLE II: DEALKYLATION OF AZO DYES WHEN MIXED* WITH A BROWN RICE DIET

Sample	DMB, %	MMB, %	AB, %
1. 3% DMB in cottonseed oil—fresh	100	0	0
2. 3% DMB in cottonseed oil—4 wks. o'd	98.0	2.0	0
3. 0.06% DMB—brown rice—2 wks. old	69.1	27.1	3.8
4. 0.06% DMB—brown rice—1 wk. old on bench in beaker covered with paper	61.4	31.3	7.3
5. 0.06% DMB—brown rice—mixed and extraction started within 20 min.	84.2	13.4	2.4
6. 0.06% DMB—brown rice heated at 90° C. for 5 days prior to mixing—extraction started within 20 min.	99.1	0.9	trace
	% DEB		% AB
7. 0.06% DEB—brown rice—1 wk. old	100		0
	% DE-ol-B		% AB
8. 0.06% DE-ol-B—brown rice—1 wk. old	100		0

* Dyes were dissolved in cottonseed oil (3% sol.) and mixed with ground brown rice to give a 0.06% concentration of the dyes.

The observation that DEB, a non-carcinogen (at least when fed to rats on a brown rice diet), is deethylated (5) and yields the same concentration of AB in tissue and blood cells as do the *N*-methyl derivatives made it of interest to study *N,N*-diethanol-*p*-aminoazobenzene (DE-ol-B), also noncarcinogenic under the same conditions.

The results are summarized in Table III. The earlier results obtained with DEB are included for comparison.

TABLE III: THE ACCUMULATION OF AB IN THE BLOOD CELLS OF RATS FED DEB AND DE-OL-B

Number of days on diet	AB from DEB γ/ml. cells	AB from DE-ol-B γ/ml. cells
2	11.5	..
6	..	0.9
6	..	1.1
7	43	..
14	..	5.2
14	..	0.5
15	28	..
17	34	..
21	31	..

Only small amounts of AB were found in the blood cells of the rats fed DE-ol-B in contrast to the results obtained with DMB and DEB. Apparently, the rat does not split the N-C_2H_4OH linkage as readily as the N-CH_3 or N-C_2H_5. This suggests that the mechanism of removal of $-C_2H_5$ groups does not involve an oxidation to $-C_2H_4OH$.

The destruction of the large amounts of DMB and AB (10) in the rat and the accumulation of AB in the blood cells of rats fed DMB suggested that it might be possible to measure the rate of destruction of DMB and AB by liver slices *in vitro*. It was thought possible to demonstrate a conversion of DMB to AB in these *in vitro* experiments. However, the results shown below (Table IV) indicate that under the conditions of these experiments AB is destroyed (metabolized) *in vitro* at least as rapidly, if not more so, as is DMB. When DMB was destroyed by the liver slices *in vitro*, no AB could be detected. No attempt has been made to determine the nature of the *in vitro* metabolism, but as only basic compounds are extracted in the procedure used it would indicate either that the azo linkage was reduced, or that the molecule was oxidized to a phenolic compound which would not have been extracted.

TABLE IV: AMOUNTS OF AZO COMPOUNDS DESTROYED ON INCUBATION OF 50 γ WITH 200 MGM. OF TISSUE SLICES AT 37.5° C. FOR 90 MINUTES

Compound	Tissue	No. of exper.	Average destroyed in γ	Range in γ
DMB	Normal liver	5	34.6	29.0-40.8
DMB	Liver tumor*	5	11.6	5.0-16.7
AB	Normal liver	5	38.4	31.3-44.8

* Classified as cholangiomas on histologic examination by Dr. S. Spitz.

In view of the fact that normal liver tissue destroyed DMB readily, it was of interest to determine whether liver tumors produced by this agent possessed this capacity and if so to what extent. The results included in Table IV indicate that the liver tumors studied were able to destroy DMB, but, on an equivalent wet weight basis and under the same conditions, did so to a significantly less extent. The average destruction of DMB and AB by 200 mgm. of normal liver slices was 34.6γ

and 38.4γ, respectively. The tumor slices (200 mgm.) destroyed 11.6γ of DMB under the same conditions.

In several experiments in which 50γ of DMB was incubated with blood cells only a small amount (5 per cent) was destroyed and small amounts (approximately 2γ) of MMB and (ca. 1γ) AB were recovered.

DISCUSSION

While the data presented above are of interest in connection with the metabolism of closely related azo dyes, which vary tremendously in their capacity to produce a neoplasm in rat liver under standard conditions, little light is thrown on the mode of action of the carcinogenic ones. The finding that the *N,N*-diethyl derivative, non-carcinogenic under the conditions of our test, leads to the same accumulation of AB in the tissues as the *N,N*-dimethyl, *N*-monomethyl and AB suggests that this dealkylation pathway may not be directly related to the problem of carcinogenesis. The observations that the administration of a methyl acceptor compound, guanido-acetic acid, is without effect on the production of liver tumors by MMB (8), and that *o*-aminoazotoluene, in which the amino group is unsubstituted, is carcinogenic, are also consistent with this view. The recent observations of Kirby (7) that *p*-aminoazobenzene may under certain dietary conditions produce liver tumors in the rat, and our observation (13) of one tumor (427 days) in a rat fed 4′-methyl-*p*-aminoazobenzene are two examples of other *p*-aminoazobenzene compounds with unsubstituted amino groups that have produced liver cancer.

While the *in vitro* experiments dealing with the destruction of DMB are by no means adequate to evaluate the rate of destruction, a calculation based on the data available, assuming the total liver weight to be 4 gms., reveals that the liver would destroy approximately 11,000γ of DMB per 24 hour period. Actually, on tumor producing diets, the rats eat about 6,000γ per day. The failure to find any AB when DMB was incubated with the liver slices raises the problem as to whether these results can be extrapolated to the intact animal, or whether possibly the small amounts of AB found *in vivo* in the rat, chiefly in the blood cells, may be due to extrahepatic demethylation. The results obtained with the liver tumors (cholangiomas) should not be interpreted as evidence of a loss of hepatic cell ability to destroy DMB. The cholangiomas are presumably derived from bile duct epithelial cells and hence any comparison would have to be between these two types of tissue. The tissue slice technic is obviously not suitable for determining the ability of normal bile duct epithelial cells to destroy DMB.

The finding that brown rice 'catalyses' the demethylation of DMB necessitates further quantitative study of the influence of the addition of protective supplements on the stability of DMB in this diet. Preliminary experiments have indicated that the inclusion of 15 per cent yeast does not influence this phenomenon. The observation that heating the rice renders the DMB much more stable and the observations of the Wisconsin group (9) indicating stability of DMB in their basal diets without rice, suggests that the presence of a 'catalyst' in the rice is responsible for this effect.

This further complication in the use of a brown rice diet, in addition to rice variation and the presence of unknown factors, makes it desirable to discard the use of the brown rice for further nutritional experiments with the azo dyes. Further use is not warranted although its use permitted: (a) The discovery of the carcinogenic action of the azo dyes (15); (b) The demonstration that the incidence of tumors in rats can be greatly decreased by the addition of crude dietary supplements such as yeast (1), and liver (11); (c) The first demonstration of the protective effect of a pure compound, riboflavin (6).

SUMMARY

1. In agreement with earlier observations it has been found that when *N,N*-dimethyl-*p*-aminoazobenzene is administered orally to rats the bulk of the azo compound found in the blood and tissues is *p*-aminoazobenzene. However, this represents only a very small portion of the *N,N*-dimethyl-*p*-aminoazobenzene fed.

2. The recovery of relatively large amounts of *N*-methyl-*p*-aminoazobenzene and *p*-aminoazobenzene from the stomach contents led to the finding that when *N,N*-dimethyl-*p*-aminoazobenzene in cottonseed oil is mixed with ground brown rice some demethylation occurs. Heating the brown rice for 5 days prior to mixing inhibited the demethylation.

3. Feeding *N,N*-diethanol-*p*-aminoazobenzene, in contrast to *N,N*-diethyl-*p*-aminoazobenzene, led to the accumulation of only small amounts of *p*-aminoazobenzene in the blood cells.

4. Liver slices under the conditions of our experiments have been found to destroy both *N,N*-dimethyl-*p*-aminoazobenzene and *p*-aminoazobenzene. No conversion of *N,N*-dimethyl-*p*-aminoazobenzene to *p*-aminoazobenzene by liver slices was observed *in vitro*.

5. Under the conditions of our experiments liver tumor slices destroyed only one-third as much *N,N*-dimethyl-*p*-aminoazobenzene as did the normal liver tissue.

ACKNOWLEDGEMENT

We wish to thank Dr. M. L. Crossley of the American Cyanamid Co. for the azo compounds used in these experiments.

REFERENCES

1. Ando, T. Experimentelle Lebenkarzinomentstehung und Getreide. Gann, **32**:252-258. 1938.
2. Elson, L. A., and Warren, F. L. The Metabolism of Azo Compounds. 1. Azobenzene. Biochem. J., **38**:217-220. 1944.
3. Elson, L. A., and Warren, F. L. Conditions Affecting Malignant Growth. British Empire Cancer Campaign, Annual Report. 1945, pp. 21-22.
4. Hashimoto, T. Über den Abbau von o-Amidoazotoluol im Tierkörper. Gann, **29**:306-308. 1935.
5. Kensler, C. J., Magill, J. W., Sugiura, K., and Rhoads, C. P. Deethylation of *N,N*-Diethyl-*p*-Aminoazobenzene in the Rat. Arch. Biochem., **11**: 376-377. 1946.
6. Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. F., and Rhoads, C. P. Partial Protection of Rats by Riboflavin with Casein against Liver Cancer Caused by Dimethylaminoazobenzene. Science, **93**:308-310. 1941.
7. Kirby, A. H. M. Carcinogenic Effect of Aminoazobenzene. Nature, London, **154**:668-669. 1944.
8. Miller, E. C., and Baumann, C. A. The Carcinogenicity of *p*-Monomethylaminoazobenzene in Various Diets and the Activity of this Dye Relative to *p*-Dimethylaminoazobenzene. Cancer Research, **6**: 289-295. 1946.
9. Miller, J. A., and Baumann, C. A. The Determination of *p*-Dimethylaminoazobenzene, *p*-Monomethylaminoazobenzene, and *p*-Aminoazobenzene in Tissue. Cancer Research, **5**:157-161. 1945.
10. Miller, J. A., Miller, E. C., and Baumann, C. A. On the Methylation and Demethylation of Certain Carcinogenic Azo Dyes in the Rat. Cancer Research, **5**:162-168. 1945.
11. Nakahara, W., Fujiwara, T., and Mori, K. Inhibition Effect of Yeast Feeding on the Experimental Production of Liver Cancer. Gann, **33**: 57-65. 1937.
12. Stevenson, Elizabeth S., Dobriner, K., and Rhoads, C. P. Metabolism of Dimethylaminoazobenzene (Butter Yellow) in Rats. Cancer Research, **2**:160-167. 1942.
13. Sugiura, K., Halter, C. R., Kensler, C. J., and Rhoads, C. P. Observations on Rats Fed with Compounds Related to Dimethylaminoazobenzene. Cancer Research, **5**:235-238. 1945.
14. Sugiura, K., and Rhoads, C. P. Experimental Liver Cancer in Rats and Its Inhibition by Rice-Bran Extract, Yeast, and Yeast Extract. Cancer Research, **1**:3-16. 1941.
15. Yoshida, T. Experimentelle Beitrag zur Frage der Epithelmetaplasie. Virchows Arch. f. path. Anat., **283**:29-240. 1932.

A Study of Three New Duck Variants of the Rous Chicken Sarcoma*

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(Received for publication October 22, 1946)

Past studies have disclosed the fact that the virus of the Rous sarcoma of chickens can be adapted to alien species of birds provided, first, that young or newborn individuals be injected with the tumor material (2, 3) and second, that this tumor had been grown in chickens of a certain age (4). By fulfilling these requirements, several other duck strains of chicken tumors have been obtained. Three of them, variants of the Rous sarcoma, will be here described because they show interesting features differing from those of the sarcoma strains previously obtained from the same Rous tumor.

The method followed consisted of injection into the breasts of newborn ducklings of 3 cc. of a cell suspension of the chicken tumor at 1:5 in saline, and of analogous passages into other ducks of the tumors induced in the foregoing passage, using in these passages the same or a much smaller inoculum. Filtrates were also used. The original chicken tumor, as well as duck tumors from different passages were also tested on chicks and chickens by injecting from 0.5 to 3 cc. of cell suspensions in the breast, or 1 cc. or more of filtrate in either the breast or the vein. Adaptation of the virus to ducks was first recognized by gross and microscopic changes in the primary tumors, by the appearance of generalized lesions, and by the ability to infect older ducks with tumor material. The strains are designated 14(e), 14(d)11, and 14(d)7.

Strain 14(e).—The donor host was a chicken 3 months old injected 13 days before with a cell suspension of the Rous sarcoma. This is the youngest chicken in which a tumor grew which proved to be most easily adaptable to ducks. Filtrates and cell suspensions of this tumor were found to be very active for chicks and chickens.

The tumor grew in the first passage in 16 of the 18 ducklings injected and regressed only in one case. Signs of adaptation were manifest in 13 of the birds. The rather amorphous chicken tumor changed into a grape-like type of growth consisting of round, solid, well-encapsulated tumor nodules mingled with similar nodules but filled with blood. From these primary lesions generalization occurred, as shown by the presence of hemorrhagic lesions in liver, spleen, bone marrow, lungs, kidneys, and also by the presence of occasional tumors in lungs and spleen. The ducks died within 14 to 34 days after inoculation. Separate lines were started from 7 of the tumors and 3 of them were carried through 3, 4, and 8 passages in ducks, being then purposely discontinued. The other 4 lines were lost because of accidental death or because the ducks employed were too old. Growth was always successful whenever ducks aged 1 or 2 days were inoculated, while it was far more irregular in older ducks. In the course of the passages, the tumors and generalized lesions showed the same characteristics as in the first passage. Death occurred within 13 to 30 days. Microscopically, the tumors were very pleomorphic sarcomas, although a large, distorted type of cell was predominant. Collagen was scanty or absent. Periosteal and endosteal tumors were observed in one case. The hemorrhagic lesions were observed, in general, independent of neoplasia.

Starting from a duck tumor of the third passage, the strain was maintained through chicks and pullets in 3 successive passages. Twelve out of 16 birds injected with cell suspensions in the breast, and 5 out of 6 injected with filtrates in the same location developed tumors. However, generalization from these tumors was negligible since only 1 of the animals developed a metastasis in the liver. At the end of the passage through chickens the tumor grew successfully in 3 out of 4 ducklings inoculated.

Strain 14(d)11.—The age of the donor chicken was 20 months and it had been inoculated with the tumor

* This investigation was carried out with the aid of a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

44 days before. This is the oldest chicken ever found furnishing a tumor very easily adaptable to ducks. Filtrates and cell suspensions of this tumor proved to be very active for chicks.

The tumor grew very rapidly in all of the 4 ducklings of the first passage, and signs of adaptation were clear in 3 of the birds. They died within 28 to 34 days with large primary tumors and a pronounced hemorrhagic disease of the liver, and one of these tumors was maintained in 4 passages through ducks, the line being then purposely discontinued. The fourth duckling of the first passage died 7 days after inoculation with a large, viscid growth very much like the original chicken tumor and it showed no generalization. In the second passage, this tumor grew rapidly in all the 6 ducklings injected, again without signs of adaptation. These signs, though, appeared at the third passage in the form of grape-like tumors, as in strain 14(e), and with the development of generalized hemorrhagic lesions and also tumors. Passages by means of cell suspensions or filtrates were continued and the line is now at its 20th passage. In the course of transplantation, the grape-like tumors soon disappeared to be replaced by rather amorphous, viscid growths of an extremely invasive type which rapidly infiltrated the skin and penetrated the abdominal cavity. Generalized hemorrhagic lesions with or without association with tumors were frequently observed in the viscera, and in one case there developed periosteal and endosteal tumors. Death occurred in from 10 to 20 days after inoculation with or without generalized lesions grossly visible.

Microscopically, the growths were rather uniform with long attenuated fibroblasts as the dominant cell type. Collagen was scarce or absent. Mitoses were rare. The hemorrhagic lesions in general were not associated with neoplasia.

The tumor always grew when ducks from 1 to 4 days of age were inoculated. It occasionally regressed in ducks 4 or 5 weeks old, and it grew but slowly and without inducing generalization in ducks 6 or 18 months old.

In 5 different passages, cell suspensions were injected into 14 chickens from 2 weeks to 3 months of age, with growth ensuing in all but one case. Filtrates were injected into 10 chicks, with positive results in all. In 3 occasions these chick-grown tumors were maintained through these hosts for 4 and 5 passages by means of cell suspensions. Growth was always successful and was often followed by generalization of the same type as seen in ducks. Filtrates were also active. At the end of the chick passages the tumors grew in ducks just as well as before these passages.

Strain 14(d)7.—The age of the donor chicken was 14 months. The bird had been inoculated with the tumor 43 days before. Cell suspensions of this tumor were effective in all of 7 chicks injected, but filtrates proved to be inactive in 2 other chicks.

The tumor grew rapidly in 4 of the 7 ducks injected, adaptation being manifest in one of the birds by the same signs as seen in the 2 tumors previously described. From this tumor a line was obtained which was discontinued after 3 passages. From 2 other tumors, lines were also obtained; one lasted 4 passages, while the other is now in its 20th passage. The ducks of the first passage died within 14 to 35 days. Like strain 14(d)11 the tumor soon became amorphous and very invasive. Survival time shortened rapidly so that quite frequently the ducklings injected with suspensions of tumor cells died in 8 or 10 days with or without generalization and, in some cases, even within 5 days, sizeable tumors had already been present. Generalization in viscera was quite frequent, with a frank predominance of hemorrhagic lesions. In fact, this was the most active strain in this respect. Periosteal and endosteal growths were quite common after a few passages, and often the bones of all the extremities were affected. However, the most interesting feature shown by strain 14(d)7 was the induction of hemorrhagic lesions in the central nervous system of a rather large number of ducklings. These lesions appeared either as a generalization from a primary tumor or following intravenous injections of filtrates, and they were observed grossly unassociated with neoplasia. A detailed report of the phenomenon, together with other pertinent experiments, will be given in another publication. Microscopically, the tumors were pleomorphic, like those induced by strain 14(e). Collagen was also scant or absent, and mitoses were rare.

Despite its extreme malignancy, no growth or growth followed by regression was observed in 5 ducks of from 6 to 18 months of age inoculated with cell suspensions. In general, the tumor grew constantly and steadily in ducks of from 1 to 30 days of age, although periods of poor growth, even in these young hosts, were encountered.

In 7 different passages a total of 38 chicks and 6 adult chickens were injected with filtrates of neoplastic or hemorrhagic lesions. Positive results were obtained in 22 chicks and 4 chickens. Cell suspensions were constantly effective in both chicks and chickens. On two different occasions the duck-grown tumors were easily kept for 5 successive passages through chicks and chickens, provided the donor host was young. With tumors from old hosts, growth was achieved in only 7 of 29 chicks and chickens injected. Generalization in viscera occurred in chicks as in ducks the same as periosteal and endosteal tumors. It is of interest to point out that in the latter case new formation of

osteoid tissue was never observed in chicks or ducks as well.

Further study of the strain disclosed the following fact of interest: At the 19th passage, 2 chickens 8 months old were injected intradermally with filtrate of a duck tumor. One of the birds, killed 60 days later, showed a sarcoma in the injected site 4 × 2 × 2 cm., conspicuous lung metastases, a large embryonal nephroma in the left kidney, and 2 smaller ones in the opposite kidney. The primary tumor showed histological signs of regression. Injection of cell suspensions of the primary tumor into 4 chicks and pullets resulted in tumors which later regressed, but injection of a similar material from the embryonal nephroma in another 4 chicks and pullets resulted in large sarcomas, one of which was maintained in chickens through 6 successive passages by cell suspensions and filtrates.

The other chicken injected with the filtrate of the duck tumor also showed a local sarcoma 4 × 3 cm. and lung metastases. The primary tumor was carried in chicks through 6 successive passages.

Leaving aside the point concerned with the significance of the kidney lesions, the point of interest is that the virus from the tumors of the 2 chickens had lost to a large extent its former capacity to infect ducks, for in each of the 2 serial passages through chicks, ducks were also injected with the following results: In the line from the embryonal nephroma, no growths were obtained in 20 ducks of from 1 to 30 days of age injected with cell suspensions or filtrates, and in the line from the local sarcoma, only 12 ducks a few days old out of 37 injected with cell suspensions developed tumors, but these growths were well localized and never induced generalized lesions.

DISCUSSION

In addition to individual characteristics, the 3 variants of the Rous sarcoma show some common traits which are different from those shown by all or some of the 6 variants of the same sarcoma obtained in 1941 and reported the following year (2).

The most important difference is that in the variants here described, despite the adaptation of the virus to ducks, their affinity for the original host, the adult chicken, was kept and it was possible to maintain the strains by passages through both chickens and ducks indifferently.[1] Other differences were the lack of affinity of the virus for the skin and intestinal tract, the amorphous character and absence of collagen in the tumors, and the lack of production of osteoid tissue following the development in chicks of periosteal and endosteal tumors. Since both the strains of 1941 and those here described were obtained from the same strain of Rous sarcoma, these findings may still be another proof (4) of the variability of the tumor virus, a variability which is not manifested in routine passages through the homologous host but only when heterologous hosts are infected.

The lack of neoformation of osteoid tissue in the chicks that developed periosteal and endosteal tumors is of interest since in some of the 1941 strains it was observed that the bone condition *osteopetrosis gallinarum* (1-3, 7) arose as a *late* lesion in chickens infected with viruses inducing this neoformation of bone. Osteopetrosis was never observed in the chickens injected with viruses of the duck variants of the Rous sarcoma here studied, and in this respect these viruses behaved like those inducing some other spontaneous chicken tumors (5). These observations tend to support the idea that transmissible osteopetrosis is not a common response to many unrelated stimuli but rather a specific lesion associated with infection by leukosis and tumor viruses.

Strain 14(d)7 deserves additional comment. In the first place, it is the most malignant variant ever obtained, sometimes killing the host in as short a period as 5 days without visible generalization but with sizeable tumors. This property was shared to a certain extent by the other duck variants of both 1941 and 1945, and may be an indication of a toxic effect. Therefore, the process of variation has resulted in a pronounced increase in the virulence of the tumor virus. However, despite this virulence, strain 14(d)7 and the others as well are but little effective in older ducks, because of the development of an age resistance by these hosts. Strain 14(d)7 was also the most active in inducing hemorrhagic lesions, and stands as a unique example in the sense that it induced such lesions in the central nervous system of ducklings. Still another property of the strain is its pronounced activity for full-grown pigeons, in which hosts malignant tumors followed by generalization were produced by cell suspensions. These latter two properties will be dealt with in separate publications.

A phenomenon of interest, so far only observed with the same strain, 14(d)7, is that after tumors were produced in 2 full-grown chickens by means of filtrates, the virus evidently varied again in these hosts because it lost its power to infect ducks older than a few days, and at best produced in new-born ducklings only rare

[1] This does not mean that the disease in chickens was the same as that induced by the original Rous sarcoma virus as attested, for instance, by the frequent development of periosteal and endosteal sarcomas in chicks injected with virus from strain 14(d)7 or by the absence of generalized lesions in those injected with virus from strain 14(e).

and well-localized tumors,[2] never followed by generalization. Indeed, events were as if the ducklings had been injected with the Rous tumor grown either in very old or in very young hosts (4). Therefore, it would seem that the virus reverted to a chicken type, but whether or not that virus was exactly the same as the original Rous virus cannot be said.

In both the tumors here studied and in those induced in 1941 by cell suspensions, the frequency of cases in which signs of adaptation were observed in the ducklings of the first passage can be taken as an indication of the number of virus units which have previously mutated or have been selected in the chicken tumor employed for inoculating the ducklings. In strains 14(e) and 14(d)11, practically all of the ducks of the first passage showed signs of adaptation of the tumor, whereas, in strain 14(d)7 as well as in some of the 1941 strains, signs of adaptation were not clear until the tumor had been carried in several passages through ducks. One can presume that during these passages the units of virus which had retained their original nature as inductors of the chicken tumor had been progressively eliminated.

Finally, what has been learned from all the duck variants of the Rous sarcoma so far studied emphasizes the fundamental instability of the viruses of avian cancer which, through a process of variation, can induce a practically limitless number of strains, each different from the others.

SUMMARY

Three new variants in ducks of the Rous sarcoma of chickens were obtained. Because they showed characteristics different from those of the variants previously studied, the strains are described in some detail. The most important difference was that the viruses of the strains here studied, despite becoming adapted to ducks, did not lose their affinity for their original host, the chicken. Also, the tissue affinities of the viruses, the amount of collagen in the tumors, and the reaction of bones of chicks to periosteal and endosteal tumors were different. The virus of one of the variants, strain 14(d)7, was of special interest for the following reasons: first, because after infecting adult chickens it lost its acquired affinity for ducks and reverted to a chicken tumor type; second, because it induced malignant tumors in pigeons of all ages; and third, because it showed an affinity for the central nervous system of young ducks.

[2] The fact that in 1 of the 2 chickens an embryonal nephroma was found from which a line of sarcoma was obtained can simply be interpreted as a localization of the virus in an already present kidney tumor. However, one has to keep in mind other possibilities discussed elsewhere (6).

REFERENCES

1. Burmester, B. R., Prickett, C. O., and Belding, T. C. A Filtrable Agent Producing Lymphoid Tumors and Osteopetrosis in Chickens. Cancer Research, **6**:189-196. 1946.
2. Duran-Reynals, F. The Reciprocal Infection of Ducks and Chickens with Tumor-Inducing Viruses. Cancer Research, **2**:343-369. 1942.
3. Duran-Reynals, F. The Infection of Turkeys and Guinea Fowls by the Rous Sarcoma Virus and the Accompanying Variations of the Virus. Cancer Research, **3**:569-577. 1943.
4. Duran-Reynals, F. The Age Factor in Adaptability of a Sarcoma Virus to Other Animal Species. Science, **103**:748-749. 1946.
5. Duran-Reynals, F., and Shrigley, E. W. A Study of Five Transplantable Chicken Sarcomas Induced by Viruses. Cancer Research, **6**:535-544. 1946.
6. Duran-Reynals, F. On the Transplantability of Lymphoid Tumors, Embryonal Nephromas and Carcinomas of Chickens. Cancer Research, **6**:545-552. 1946.
7. Jungherr, E., and Landauer, W. Studies on Fowl Paralysis. A Condition Resembling Osteopetrosis (Marble Bone) in the Common Fowl. Storrs Agr. Exp. Sta. Bull., **222**:1-34. 1938.

Transmission to Adult Pigeons of Several Variants of the Rous Sarcoma of Chickens*

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(Received for publication October 22, 1946)

Previous studies have shown that both cell suspensions and filtrates of the Rous sarcoma grown in adult chickens were entirely inactive when injected into even newborn pigeons (2). On the other hand, it was also shown that the Rous virus can be adapted to ducks and other species as well, provided the recipient host be a young or a newborn individual (1, 2) and the donor host be endowed with a certain degree of resistance linked with age (4).

In the present publication, we report the fact that the Rous sarcoma after adaptation to ducks can be easily transferred to pigeons with little or no age limitations on the part of the recipient host. The duck variants of the Rous sarcoma studied were strains HV obtained in 1941 (1), 14(e), and 14(d)7 obtained in 1945 (5), and another duck tumor strain 55(e), the origin of which is rather uncertain. The pigeons employed were of the common varieties found in markets.

Additional attempts to transfer the Rous sarcoma to pigeons. About 15 pigeons, from a few weeks to 4 months of age, were injected in the breast muscles with various amounts of cell suspension and filtrates of the Rous sarcoma from chickens of different ages. Again, no success was achieved. In one case only a tumor nodule 0.3 cm. in diameter was found in the injected site 40 days after inoculation of 2 cc. of cell suspension at 1:5. This tissue grafted into 3 more pigeons induced no growths.

Strain 14(e).—Three pigeons 2 months old were injected in the muscles of each breast with 1 cc. of cell suspension of a duckling tumor of the third passage. Tumors developed which attained a sizable growth after 30 days, but regressed in 2 of the pigeons. The bird bearing the third tumor measuring by now 4 × 3 × 2 cm. was killed 73 days after injection and was passed by means of cell suspensions into 4 other pigeons. Growth followed in 3 with regression in 2 of them. The third pigeon, bearing a small tumor, was killed 13 days after inoculation and the tumor was passed into 2 other pigeons. Again tumors grew which later regressed. The tumors were rather infiltrating but no generalization was observed. No microscopic studies were carried out. The loss of the strain prevented more detailed investigations.

Strain HV.—At the 42nd passage of the strain, 2 pigeons about 5 months old were injected in each breast with 1 cc. of cell suspension at 1:5. Both animals developed tumors, one of which attained a size of 2.5 × 2.5 × 2 cm., while the other was smaller. The first animal was killed 22 days after inoculation and cell suspensions from the tumor were injected into 2 pigeons, while filtrates were injected into 2 others. The cell suspensions induced growths which were followed by regression, while filtrates were ineffective.

The tumor in the second pigeon remained stationary for several weeks and the bird was killed 3½ months after inoculation. A few small nodules of tumor tissue were still present. A cell suspension of this tissue inoculated into 2 pigeons induced no growth.

The same experiment was repeated with a tumor of the 44th passage of the duck strain and the same results were obtained.

The tumors in pigeons were lobulated, translucent, soft, and viscid, well circumscribed from the adjacent tissues, and they never induced generalized lesions. No microscopic examinations were carried out. The loss of the duck tumor strain prevented further studies.

Strain 55(e).—A brief description of how this strain was obtained has been given elsewhere (3). A cell suspension from a tumor induced in a pigeon by means of methylcholanthrene was inoculated into 4 ducklings with the result that tumors developed rapidly in all the animals injected. These tumors were easily transmitted to other ducklings in 12 successive passages by means of cell suspensions or filtrates. Although on the one hand, strain 55(e) offered many analogies with several

* This investigation was aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

FIG. 1.—Tumor induced in 24 days in pigeon injected at age of 6 weeks with cell suspension from duck tumor of strain 55(e).

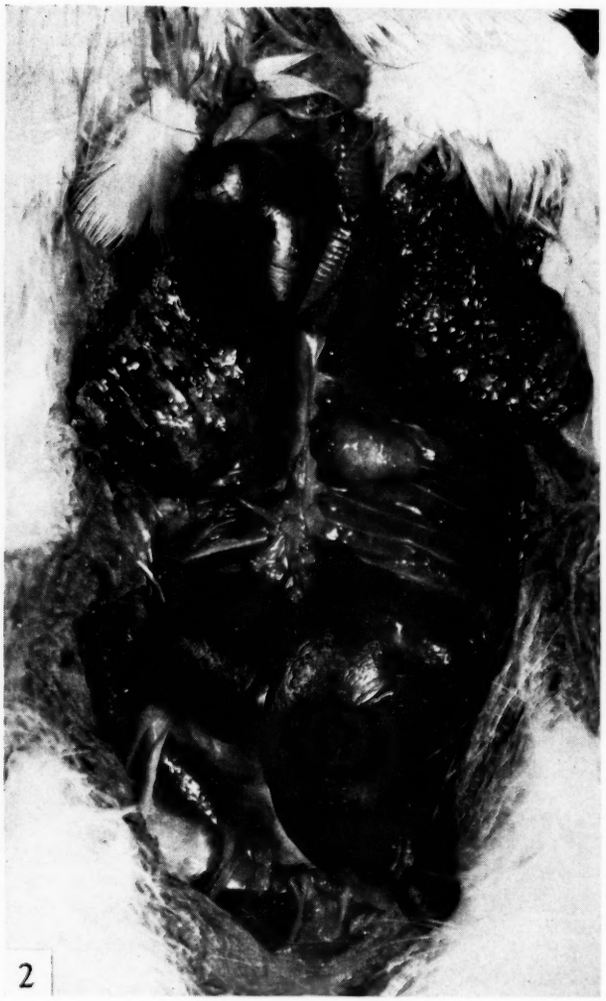

FIG. 2.—Metastases in lungs, liver, and one rib following primary tumor in 1 year old pigeon injected in breast with cell suspension from duck tumor of strain 14(d)7. The bird died 27 days after inoculation.

duck variants of the Rous sarcoma, which, by that time, were kept in the laboratory, on the other hand, it had characteristics of its own, and at the present moment we are unable to state how the strain originated. Of interest in the experiments to be described is the fact that the primary tumors both grossly and microscopically were practically identical to those of strains A, HV, and HC (1).

In 10 different passages in the course of transplantation of this strain, each of 25 pigeons about 6 weeks old was injected in the breast muscles with 1 cc. of cell suspension of the duck tumor grown in young hosts. Growth followed in 17 cases, in 8 of which regression occurred. In 4 other pigeons injected with filtrates, no lesions developed.

The tumors grew at a moderate rate, but steadily, and attained in some cases a size of 6 × 4 × 3 cm. after 2 or 3 months before regression or the death of the host. These tumors in pigeons resembled very much the duck-grown tumors. They were lobulated, translucent, resilient, or soft (Fig. 1). Microscopically they proved to be largely collagenous with many extremely loose areas. However, they were very well circumscribed from the adjacent tissues and generalization never occurred. Death occurred in 3 cases, while in the other cases the pigeons were killed for transplantation or other purposes.

A second passage was attempted on 3 occasions by injecting cell suspensions into other young pigeons. In one instance, growth followed by regression was observed in all of the 4 pigeons inoculated, while no lesions developed in the other two cases. However, the same cell suspensions injected into ducks produced the customary disease with large primary tumors followed by

generalized neoplastic and hemorrhagic lesions, and filtrates of these tumors were also active in other ducks, thus proving that free virus was present in them.

Strain 14(d)7.—At the seventh passage of the strain, 3 pigeons 12 months old were injected in the muscles of each breast with 2 cc. of cell suspension at 1:5 from a large tumor 7 days old grown in a duck 15 days old at death. Growth followed in the 3 pigeons. In one of them the tumor remained stationary for about 3 months and finally regressed. The second pigeon died in 27 days with a rather necrotic but very infiltrating primary tumor filling the whole breast, and hemorrhagic metastases in lungs, liver, and one rib (Fig. 2). Filtrates from the primary tumor of this bird proved ineffective in 4 young pigeons. The third pigeon was killed 14 days after injection. There was a tumor 3 × 3 × 3 cm. in each side of the breast and many minute metastases in the liver. A second passage by cell suspension into 2 pigeons resulted in 2 tumors, one of which regressed. The remaining bird was killed 27 days after injection and was found to have a large, firm tumor wholly free of necrosis. This tumor produced in a third passage a growth followed by regression in 1 of 3 pigeons inoculated with cell suspensions, but filtrates of the same growth induced no tumors in 2 pigeons 6 weeks old. At both the first and second passage a total of 6 ducklings and 2 chicks were inoculated with cell suspensions. Large primary tumors followed by generalization such as is usually found in the routine passage of strain 14(d)7 were induced in all animals.

Another line was started with a duckling tumor at the 21st passage of the tumor strain. In a first passage, 4 pigeons from 2 to 10 months old were injected with 1 cc. of cell suspension in each breast. Growths followed in all, but regressed only in the 10-month-old pigeon. Another of the pigeons died in 15 days with a large breast tumor and no metastases, while the other 2 died in 27 days with large primary tumors and many metastases in the spleen, lungs, and liver. In a second passage, cell suspensions of the tumors of one of these birds and filtrates from the tumors of another were inoculated into 7 pigeons. The cell suspensions produced temporary growths, while the filtrates were ineffective.

Microscopically, both the primary and metastatic tumors derived from strain 14(d)7 kept many of the characteristics present in ducks. The growths, in general, were loose and rather pleomorphic (Fig. 3). Other times they consisted of large cells arranged in a syncytial fashion and containing many nuclei. Mitoses were rare. The tumors, especially in the first passage, were very invasive—this characteristic being especially conspicuous in the metastases in the liver, lungs, and spleen. Here, the tissues were so diffusely invaded by the large tumor cells that this trait, together with the conspicuous lymphoid and myeloid cell reaction either close to the tumor tissue or distant from it, and the subsequent necrosis and vascular alterations, frequently gave the impression that one was dealing with inflammatory lesions. Some of the tumors of the second passage were almost unrecognizable on account of the heavy cell reaction. Another feature also encountered with tumors derived from strain HV was the presence of tumor nodules consisting of a collagenous matrix from which cells were practically absent.

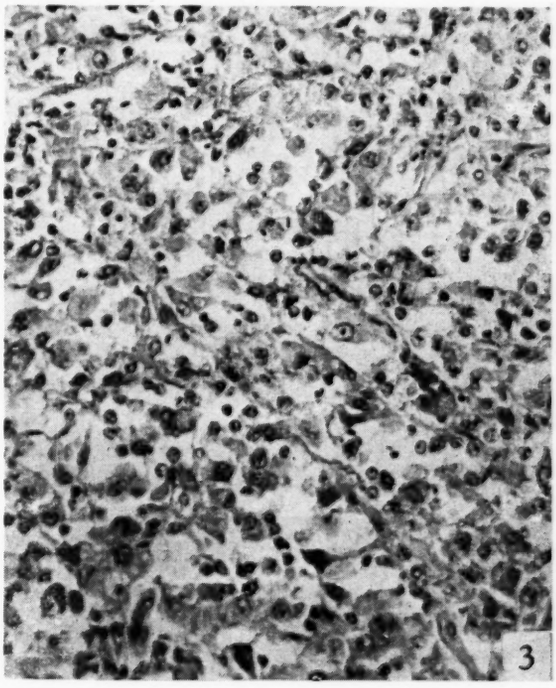

FIG. 3.—Tumor induced in pigeon 3 months old inoculated with cell suspension from duck tumor of strain 14(d)7. Mag. × 325.

DISCUSSION

The results described are of interest in what concerns the problem of species-specificity in relation to cell growth. It is well known that no restrictions to progressive growth of alien cells are present in embryos (8), newborn and young individuals (1, 2), or in certain locations of the adult, such as the brain (8) and the anterior chamber of the eye (6, 7). But with the duck variants of the Rous sarcoma here studied the case is entirely different, for tumor cells from strains 14(e), 55(e) and H V grew well locally in the breast of pigeons several months old, and on occasions the tumors thus induced also grew in pigeons in another passage. Results with another variant, strain 14(d)7, were far more interesting. Here, suspensions of tumor cells caused

fast-growing tumors to develop, followed by widespread metastases and death in pigeons as old as 12 months—the whole picture being one of an extremely malignant disease.

True, these pigeon tumors could not be maintained beyond a second or third passage, and growth in these passages was far less malignant than in the first, but as a long experience on transplanting avian tumors has shown, attempts to obtain stable lines of pigeon tumors have not been numerous enough to justify the conclusion that such lines cannot be obtained.

Concerning the results obtained with strain 14(d)7, one has to keep in mind that this is the most malignant of all avian tumors so far observed.

There would seem to be little doubt that the pigeon tumors, at least to a certain extent, are the result of multiplication of the injected cells, for filtrates of these growths tested under the same conditions as the cell suspensions failed to induce tumors. However, virus was plentiful in the growths as inoculations into ducks proved. Further experiments will show whether filtrates can also be active for pigeons under certain conditions.

SUMMARY

Suspensions of tumor cells of 2 duck variants of the Rous sarcoma and another duck tumor of uncertain origin injected into the breast of pigeons several months old induced local growths never followed by generalization, while tumor cells from another variant induced even in pigeons 12 months old large local growths often followed by generalized metastases and causing death of the host in a few weeks. These tumors have been maintained in 2 or 3 successive passages through pigeons.

REFERENCES

1. Duran-Reynals, F. The Reciprocal Infection of Ducks and Chickens with Tumor-Inducing Viruses. Cancer Research, **2**:343-369. 1942.
2. Duran-Reynals, F. The Infection of Turkeys and Guinea Fowls by the Rous Sarcoma Virus and the Accompanying Variations of the Virus. Cancer Research, **3**:569-577. 1943.
3. Duran-Reynals, F., and Shrigley, E. W. Virus Infection As an Etiological Agent of Cancer. A.A.A.S. Research Conference on Cancer, 1944, pp. 1-23.
4. Duran-Reynals, F. The Age Factor in Adaptability of a Sarcoma Virus to Other Animal Species. Science, **103**:748-749. 1946.
5. Duran-Reynals, F. Study of Three New Duck Variants of the Rous Chicken Sarcoma. Cancer Research, **7**:000-000, 1947.
6. Greene, H. S. N., and Lund, P. K. The Heterologous Transplantation of Human Cancers. Cancer Research, **4**:352-363. 1944.
7. Greene, H. S. N., and Murphy, E. D. The Heterologous Transplantation of Mouse and Rat Tumors Cancer Research, **5**:269-282. 1945.
8. Murphy, J. B. The Lymphocyte in Resistance to Tissue Grafting, Malignant Disease, and Tuberculous Infection. An Experimental Study. Monographs of the Rockefeller Institute for Medical Research, No. 21, September 20, 1926.

Carcinogenic Action of Ethyl Urethane on Rats

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With an Appendix by Rudolph Jaffé, M.D.

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(Received for publication August 9, 1946)

Recently Nettleship and Henshaw (6) observed a significant augmentation of the incidence of pulmonary adenomas of mice of the C3H strain treated by repeated injections of ethyl urethane. Henshaw and Meyer (1) confirmed this observation working with mice of the abc strain, which has a high spontaneous rate for tumors of this type. These authors have shown that the incidence of adenomas produced by the injection of urethane depends on the amount applied, and concluded that a single nonanesthetic dose is sufficient to produce some effect. Larsen and Heston (5) studied a number of narcotics with respect to their carcinogenic action. Only ethyl urethane and some other urethane derivatives were found to produce pulmonary adenomas in mice. We have found that ethyl urethane applied by injection or by mouth produced pulmonary adenomas in our noninbred strain of mice, which has a relatively low spontaneous incidence of lung adenomas (4). The action was very marked when the active substance was injected or given orally. Each author confirmed the high degree of specificity of urethane in producing only pulmonary adenomas, since no other type of tumor has been observed in the treated mice.

As pulmonary adenoma of mice is a special kind of relatively benign tumor, which very frequently occurs spontaneously, it seemed of particular interest to investigate the action of urethane on an animal species which does not develop this kind of neoplastic growth spontaneously. For this purpose rats seemed especially suited, as they seldom develop spontaneous lung tumors (2). The resistance of rat lung tissue against development of tumors has been shown also in experiments in which carcinogenic hydrocarbons have been injected intravenously. R. Jaffé (3) found only 2 pulmonary sarcomas in 90 rats injected intravenously with an oily solution of methylcholanthrene, while 30 per cent of the animals had tumors in some other site.

It therefore seemed of interest to investigate the carcinogenic action of urethane on rats. If the action of this substance is as specific as the results with mice seemed to indicate, it was hoped that some reaction of rat lungs would be produced which does not occur spontaneously.

MATERIALS AND METHODS

The rats used were from our own albino strain which shows a spontaneous tumor rate of about 5 per cent at the age of 1½ years. The spontaneous tumors observed are mostly malignant retroperitoneal or subcutaneous sarcomas, squamous epitheliomas of eyes and ears and a few cases of benign mammary adenomas. No spontaneous pulmonary tumors nor spontaneous hepatomas have ever been observed. All animals were 2 to 3 months old at the beginning of the experiments.

The urethane was administered by the oral route or injected intraperitoneally. In the first case the crystalline product was thoroughly mixed with the diet in an amount such that the food contained 0.15 per cent of urethane. The diet consisted of peanut presscake, ground corn, powdered milk, salt, and 2 per cent sesame oil, to which was added a concentrated preparation of vitamins A and D. The food was administered *ad libitum*. The animals gained weight on this diet containing urethane. It was continued until the rats died or were sacrificed. The last animals were killed after 15 months on the diet. In another series the urethane was injected; 1 cc. of a 10 per cent aqueous solution was administered to each animal by the intraperitoneal route. All the animals weighed about 100 gm. at the beginning of this experiment. The dose was sufficient to anesthetize them. The injection was repeated 30 times within 3 months. The early mortality was higher in this group than in the group that received the urethane with the diet.

Each rat that died or was sacrificed was autopsied and examined for the presence of tumors. Tissue of lung, heart, liver, kidney, and spleen was fixed in formol or Bouin solution and microscopic examination was performed in the usual manner.[1] It may be emphasized that the percentage of pulmonary adenomas reported may be a minimum number, because when the nodules were very small, it was possible to miss them in the cutting of the paraffin block. Only tumors

[1] We are indebted to Prof. R. Jaffé and Dr. J. A. O'Daly for the microscopic examination of the material. A detailed description will be published later.

that have been confirmed microscopically are reported in this paper.

RESULTS

In Table I a summary is given of the tumors observed in rats after treatment with urethane. As no tumor was found in animals that died within the first 3 months after the beginning of the treatment, we did not include them in the calculation. In the series fed a diet containing 0.15 per cent of urethane, 28 per cent developed pulmonary adenomas. If only those animals that survived one year or more are counted, the percentage of animals with pulmonary adenomas is 59. In the series receiving injections of urethane, the percentage of animals with lung adenomas was 7 in those that survived more than 3 months, and 8 in those surviving 1 year.

Microscopic lesions of a proliferative character have been observed in the lungs of a number of the treated animals. Metaplasia of the alveoli and proliferation of the bronchial epithelium have been found. In several cases these lesions existed together with real adenomas. The tumors were mostly multiple and varied greatly in size. Mostly they were about 1 mm. in diameter, in a few cases nodules of 5 mm. diameter have been found; in other cases the lesions were not visible macroscopically.

In addition to the lung tumors, we found a number of hepatomas, which must be regarded as elicited by the treatment with urethane. The percentage was less than that of the lung tumors; in the group of rats fed the diet containing 0.15 per cent of ethyl urethane only one case of an incipient and macroscopically invisible hepatoma has been observed, whereas the animals injected with that compound showed 27 per cent of hepatomas when the animals that survived more than 3 months are included. In all the cases of hepatomas observed in these animals the tumors were not visible with certainty macroscopically, although the tumor-bearing livers were of a dark color or showed some suspicious clear zones. Microscopically the tumors resembled those observed after the feeding of *p*-dimethylaminoazobenzene.

Also included in Table I are the animals of a series which had been treated identically with those receiving urethane injections, but in addition got a single intra-

TABLE I

Series no.	Treatment	Initial numbers of animals	Tumors found in animals dying at				
			3 mos.	6 mos.	9 mos.	12 mos.	15 mos.
71	Stock diet containing 0.15% urethane	57	10 negatives	8 negatives 1 bronch. prol.	8 negatives 2 bronch. prol. 2 metapl.	6 negatives 5 bronch. prol. 1 metapl. 2 pulm. aden.	2 bronch. prol. 2 metapl. 5 pulm. aden. 1 endothel. 1 sarcoma 1 hepatoma
66-B	30 Injections of 100 mgm. urethane each	28	10 negatives	2 negatives 1 hepatoma			7 negatives 3 hepatomas 1 pulm. aden. 1 sarcoma
66-A	30 Injections of 100 mgm. urethane each plus 1 i. v. injection of 2 mgm. methylcholanthrene	25	6 negatives	4 negatives 1 bronch. prol. 1 sarcoma	1 negative	1 negative 1 bronch. prol.	5 negatives 1 carc.+ bronch. prol. 2 hepatomas pulm. aden. 4 hepatomas

Tumors and proliferative pulmonary lesions observed in rats after treatment with ethyl urethane.

DESCRIPTION OF FIGURES 1 TO 6

FIG. 1.—Macroscopic aspect of lung of rat fed diet containing 0.15 per cent of ethyl urethane for 13 months. Two adenoma nodules are visible.

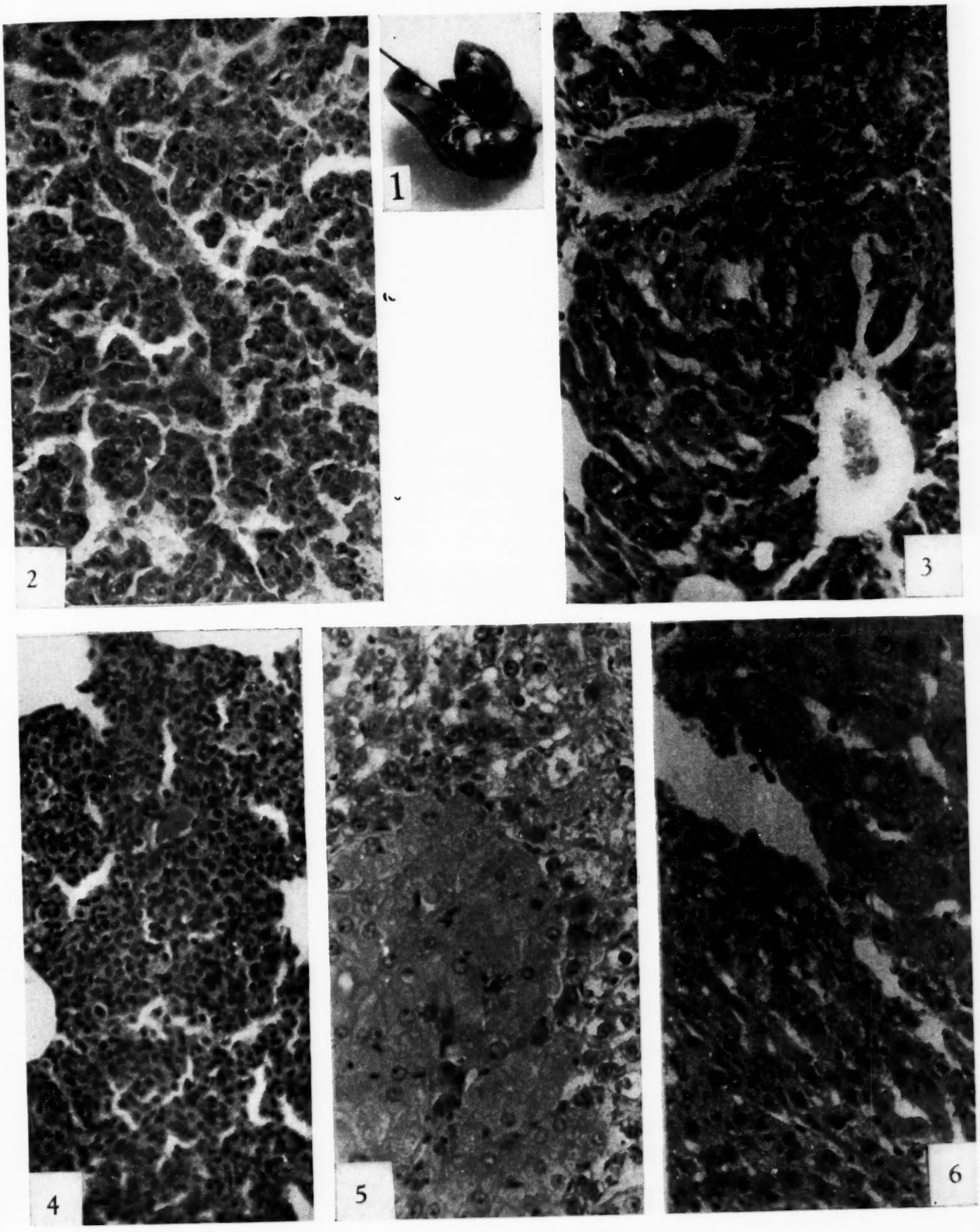
FIG. 2.—Microscopical aspect of pulmonary adenoma of rat fed diet containing 0.15 per cent of urethane for 12 months.

FIG. 3.—Microscopical aspect of lung adenoma in rat 10 months after having received 30 injections of 100 mgm. of urethane each.

FIG. 4.—Epithelial metaplasia of alveolae in rat treated with urethane injections.

FIG. 5.—Hepatoma in rat treated with 30 injections of urethane and kil'ed after 15 months.

FIG. 6.—Hepatoma in rat treated with 30 injections of urethane and 1 injection of methylcholanthrene, killed 12 months after beginning of treatment.



FIGS. 1-6

venous injection of 2 mgm. of methylcholanthrene in olive oil. Nine per cent of this group showed pulmonary adenomas compared with 7 per cent of the series which received only urethane injections. The incidence of hepatomas was 27 per cent in both groups. The size of the liver tumors in the latter series was much greater. Three animals had macroscopically visible tumors of 3 to 30 mm. in diameter. The incidence of tumors in organs other than lungs or livers was 9 per cent in this group of animal that received methylcholanthrene. In previous experiments performed in this Institute with 60 rats of the same breed and kept under identical conditions, a single injection of the same amount of methylcholanthrene resulted in a 30 per cent incidence of tumors in various organs, mostly retroperitoneal sarcomas and squamous epitheliomas (3). In the present series only one case of a squamous epithelioma and one case of a retroperitoneal sarcoma has been observed in a effectual total of 19 animals. The number of animals in this series is not sufficient to draw definite conclusions as to whether the small number of tumors caused by methylcholanthrene is due to the simultaneous application of urethane. More experiments are under way to investigate the interaction of these two carcinogens in rats.

Of the total of 16 pulmonary adenomas observed in our rats, 13 were found in males and only 3 in females. At the beginning of the experiments the number of males and females was equal, but the females showed a higher premature mortality. Of the 35 rats which survived more than 1 year after the beginning of the experiments, 32 were males. It is therefore impossible to conclude whether a sex difference exists in respect to susceptibility to the production of pulmonary adenomas. The higher mortality of the females was chiefly caused by pregnancy and miscarriage.

Besides the tumors described, a few were found that must be regarded as spontaneous; 2 were retroperitoneal sarcomas, 1 with liver metastasis, and 1 endothelioma of the liver with lung metastasis. The incidence of these spontaneous tumors is about the same as usually observed with our animals.

DISCUSSION

The experiments described above demonstrated that urethane, administered by oral or parenteral routes, produces pulmonary adenomas in a high percentage of the treated rats. The effect is more accentuated when urethane is fed with the diet, in contrast to the observation in mice, where the parenteral route was more effective. Apparently urethane has the same action in mice and rats, producing lung adenomas in both species of animals. This is remarkable inasmuch as spontaneous pulmonary adenomas are very common in mice while they have not been observed in rats. Moreover the lungs of rats are very resistant to the action of other carcinogenic agents, as has been shown with methylcholanthrene (3). Nevertheless, under the action of prolonged application of urethane, this organ shows a marked reaction. Precancerous lesions can be observed such as metaplasia of the alveoli and proliferation of the bronchial epithelium. The first real lung adenomas were observed after 1 year. They may be barely visible to the unaided eye, while in some cases we found nodules of a diameter of 1 to 5 mm. Metastasis of these tumors have never been observed in our rats.

While the only reaction observed with urethane in mice is the production of pulmonary adenomas, in our urethane-treated rats some hepatomas were found also, which must be considered as produced by this substance. The series which received injections of urethane showed more hepatomas than the animals fed the substance with their diet. In the latter group only one case of incipient hepatoma was observed, whereas of the animals that had been injected with urethane, hepatomas developed in 27 per cent. Compared with *p*-dimethylaminoazobenzene, however, the action of urethane in producing hepatomas is weak. Administered with the diet at the level of 0.015 per cent, the latter substance produces 77 per cent of hepatomas in the treated animals of our strain within 15 months, while 0.15 per cent of urethane produced 1 single incipient hepatoma in 12 treated rats which survived more than 1 year on the diet. These animals must have ingested during the experimental period at least 7 gm. of urethane, whereas the rats injected with urethane received a total amount of 3 gm. Nevertheless, they developed more hepatomas than the first group. The interpretation may be that the time factor is more important than the dose for the production of hepatomas with urethane, while in respect to the development of pulmonary adenomas this is the opposite. The first group of animals received roughly half the amount of urethane during the first 3 months that the group treated by injection received. Only more extensive comparative studies can explain the different effects of feeding and injection of urethane in rats.

One group of rats was treated with injected urethane in the same manner as the group referred to above, but moreover was injected with 2 mgm. of methylcholanthrene in olive oil intravenously. The results observed in these animals with respect to the development of pulmonary adenomas and hepatomas did not differ significantly from that obtained in the series which did not receive the carcinogenic hydrocarbon. The hepa-

tomas observed in these animals were larger than those elicited by the treatment with injected urethane only. The same amount of methylcholanthrene administered to rats in an analogous way produced tumors (sarcomas and squamous epitheliomas) in 30 per cent of the treated animals (3), while in the present series only 9 per cent of the rats had these tumors. The question, whether the production of methylcholanthrene-elicited tumors is reduced by the simultaneous application of urethane, as these results seem to indicate, will be studied in further experiments.

The observation that urethane, injected or given by the oral route, is capable of producing pulmonary adenomas and hepatic tumors in rats should be taken as a warning against prolonged therapeutic use of this substance in human beings.

SUMMARY

One series of 57 rats was fed 0.15 per cent of ethyl urethane in the diet for 15 months. Fifty-nine per cent of the animals surviving more than 9 months developed pulmonary adenomas. Moreover, 1 case of an incipient hepatoma was found.

Twenty-eight rats were injected 30 times with 100 mgm. of urethane and 7 per cent of the animals surviving more than 9 months after the first injection developed pulmonary adenomas; 25 per cent developed hepatomas within 15 months.

Twenty-five rats received 1 intravenous injection of 2 mgm. of methylcholanthrene in olive oil and 30 injections of 100 mgm. in an aqueous solution of urethane intraperitoneally; 9 per cent of these animals had pulmonary adenomas and 27 per cent had hepatomas after 15 months.

Appendix

Histological Findings in Lungs and Livers of Rats Treated with Ethyl Urethane

Rudolf Jaffé, M.D.

A short description of the histological observations made in the lungs and livers of rats treated with urethane, as described in the foregoing paper, will be given.

The lung tumors found were mostly adenomas of the same type that occur in mice spontaneously or after treatment with carcinogenic hydrocarbons or urethane. They are well limited and consist of high epithelial cells, often glandular in arrangement. The cells are pale with large, well-formed and colored nuclei. Often transitions to other cell layers may be observed, which are more solid and consist of shorter cells. The cells are situated clearly intra-alveolars, but a connection with the branches of the bronchus has never been found. They are apparently derived from metaplastic alveolar epithelial cells.

A metaplasia of the alveolar cells without tumor formation can be observed frequently. These areas of metaplasia may be found in the neighborhood of atelectatic foci of various sizes or of vegetations of the connective tissue. As the metaplastic areas can be more or less of glandular-like aspect, it is sometimes difficult to decide whether the neoformation is a tumor or not.

Vegetations of bronchial epithelium are frequently observed; they may be situated either within or exterior to the bronchi. Vegetations with glandular appearance may be found surrounding larger bronchial branches. These formations probably do not deserve to be designated as tumors, although they may be quite tumor-like in their histological aspect. They are mostly found in combination with purulent bronchitis.

Infarcts were found frequently in the border zone of the lungs. They were always typical infarcts without the epithelial vegetations found in lungs of rabbits or rats injected intravenously with methylcholanthrene (3). The artery corresponding to the infarcted area showed an endoarteritic process, which was sometimes combined with a thrombus, but never with complete occlusion. The same kind of lesion of blood vessels without infarctation could be observed also, and infarcts without these lesions have been found. The blood vessels appeared dilated only in these cases.

The hepatomas found in the livers of the rats treated with urethane showed the same histological aspect as described by Opie (7) in rats fed *p*-dimethylaminoazo-

benzene. All the hepatomas observed in the present series were derived from hepatic cells and showed various aspects of solid and glandular forms. No tumors of the bile-duct epithelium similar to those in hepatomas induced by *p*-dimethylaminoazobenzene have been observed in these cases, although a few small bile-cell cysts have been found.

REFERENCES

1. Henshaw, P. S., and Meyer, H. L. Minimal Number of Anesthetic Treatments with Urethane Required to Induce Pulmonary Tumors. J. Nat. Cancer Inst., **4**:523-525. 1944.
2. Jaffé, R. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere. Kaninchen, Meerschweinchen, Ratte, Maus. Berlin: Julius Springer. 1930.
3. Jaffé, R. Experimentos para producir tumores pulmonares. Rev. Sudamer. Morfol., **3**:91-94. 1945.
4. Jaffé, W., Jr. Producción de adenomas pulmonares en ratones por acción del uretano con consideraciones sobre la localización de tumores artificiales. Rev. Policlín. Caracas, **13**:445-452. 1944.
5. Larsen, C. D., and Heston, W. E. Induction of Pulmonary Tumors in Mice by Anesthetic Agents. Abstr., Proc. 36th Ann. Meet., Am. Assoc. for Cancer Research. Cancer Research, **5**:592. 1945.
6. Nettleship, A., and Henshaw, P. S. Induction of Pulmonary Tumors in Mice with Ethyl Carbamate (Urethane). J. Nat. Cancer Inst., **4**:309-319. 1943.
7. Opie, E. L. The Pathogenesis of Tumors of the Liver Produced by Butter Yellow. J. Exper. Med., **80**:231-246. 1944.

The Response of Rats to the Simultaneous Application of Two Different Carcinogenic Agents

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(Received for publication August 9, 1946)

The present study was undertaken to investigate the carcinogenic reaction of rats treated with two different and differently acting carcinogens. This seemed to be of interest for various reasons. First, it would be possible to study the influence of one carcinogenic agent on the action of another. Although there are numerous publications on the action of carcinogenic substances on established tumors, transplanted and spontaneous, the interaction of two differently acting chemical carcinogens has, as far as we know, not yet been studied. The second reason is that it may be possible to decide whether in the animals susceptible to the action of one carcinogen there exists a predisposition for developing also the type of tumors characteristic for the second agent applied at the same time, *e.g.* whether a rat that will develop a hepatic tumor by the action of *p*-dimethylaminoazobenzene is more susceptible to the effect of methylcholanthrene than one that resists the carcinogenic diet.

The experiments were made with albino rats of our own unselected breed, which has a relatively high incidence of spontaneous tumors. Such a strain seemed more suited for this study than a highly selected one, in which response to carcinogenic stimuli and localization of induced tumors are influenced by artificially selected genetical factors. It may be assumed certain differences in reaction to carcinogenic stimulation exist in these unselected animals, which should be revealed by the experiments. Moreover, it should be possible to observe the effect of specific carcinogenic agents on the incidence of spontaneous tumors.

MATERIALS AND METHODS

The strain of albino rats used has a spontaneous tumor incidence of 5 per cent at the age of 1½ years, chiefly of malignant retroperitoneal sarcomas, squamous epitheliomas and benign mammary fibroadenomas.

The animals were fed *ad libitum* the stock diet consisting of peanut meal, ground corn, milk powder, salt, calcium carbonate, sesame oil and a concentrate of vitamins A and D. A 0.66 per cent solution of *p*-dimethylaminoazobenzene (Merck) in sesame oil was added to this diet in the proportion of 2.25 per cent, resulting in a food mixture containing 0.015 per cent of the dye. The rats of each series were kept in a common cage. Each animal that died or that was sacrificed, was autopsied and examined for the presence of tumors. Tissue from liver, lungs, heart, kidney, spleen, and tumor was fixed in Bouin's solution and microscopic examination was performed after the material had been cut and stained in the usual manner. We are indebted to Dr. R. Jaffé for the microscopic examination of this material. The rats were 2 to 3 months old at the beginning of the experiments and gained weight during the experimental period. The carcinogenic diets were fed continuously throughout the experiments. Those animals which survived 15 months after the beginning of the experiments were killed with gas and examined as already described.

The diet given series 70 was prepared by using a solution of *p*-dimethylaminoazobenzene in cod liver oil, whereas all the other series received diets prepared with a solution in sesame oil. This modification in the first diet was made because it had been found that cod liver oil reduced the number of tumors produced by methylcholanthrene in rats (4). Series 73 was fed a diet which contained the same amount of the dye, with the addition 0.15 per cent of ethyl urethane. This substance has been found to produce pulmonary adenomas and hepatomas in rats (6). Series 67 and 70 received one intraperitoneal injection of 0.1 ml. of a 2 per cent solution of methylcholanthrene in olive oil 1 week after the beginning of the carcinogenic diet.

RESULTS

A summary is presented in Table I showing the different types of tumors observed in the various series and at various time intervals after the beginning of the experiments. It can be seen that no hepatic tumors occurred earlier than 12 months after the beginning of

the diet. Therefore, the final percentage of liver tumors was calculated on the basis of the survivors at 9 months. The incidence of hepatomas was 75, 78, 61, and 77 per cent respectively. Series 70 showed only a slight decrease but the difference is not sufficiently great to be significant. Obviously the simultaneous application of methylcholanthrene or urethane with the *p*-dimethylaminoazobenzene diet did not alter the number of hepatic tumors elicited by the latter to a significant degree.

TABLE I: TUMORS OBSERVED IN RATS TREATED SEPARATELY AND SIMULTANEOUSLY WITH VARIOUS CARCINOGENS

No.	Treatment	Initial number of animals	Tumors found in animals			
			3 mos.	6 mos.	9 mos.	12 mos.	15 mos.
68	Diet with 0.015% dimethylaminoazobenzene	28		3 negatives	1 negative	1 negative 3 hepatomas	5 negatives 14 hepatomas 1 sarc. + hepat.
67	Diet with 0.015% dimethylaminoazobenzene + 2 mgm. methylcholanthrene injected	28		1 negative 1 sarcoma	2 sarcomas 1 epithelioma	1 negative 2 sarc. + hepat. 3 hepatomas	3 negatives 6 sarc. + hepat. 1 epithelioma 7 hepatomas
70	Same, but diet prepared with cod liver oil	27			3 negatives 1 sarcoma	4 negatives 1 sarcoma 1 mammary adenoma 1 mam. ad. + hepat.	2 negatives 1 sarcoma 1 sarc. + hepat. 12 hepatomas
73	Diet with 0.015% dimethylaminoazobenzene and 0.15% ethyl urethane	24	1 neg.	4 negatives	2 negatives	2 negatives 2 hepatomas 1 pulm. adenoma 1 pulm. aden. + hepat.	1 sarcoma 8 hepatomas 1 pulm. ad. + hep. 1 kidney ad. + hep.
71	Diet with 0.15% ethyl urethane (Controls)	57	10 neg.	9 negatives	12 negatives	12 negatives 2 pulm. adenomas	4 negatives 5 pulm. adenomas 1 hepatoma 1 sarcoma 1 endothelioma
12	2 mgm. methylcholanthrene injected (Controls)		2 neg. 1 sarc. 1 epith.	5 negatives 4 sarcomas 1 epithelioma	7 negatives 1 sarcoma 2 epitheliomas	10 negatives 2 sarcomas 2 epitheliomas 1 liver sarcoma	10 negatives 3 sarcomas 3 epitheliomas

Series 67 had a tumor incidence of 46 per cent when hepatomas are not included in the calculation. These tumors must be regarded as produced by the injection of methylcholanthrene. Of 54 rats maintained under identical conditions and injected with the same amount of methylcholanthrene, but not receiving the carcinogenic diet, 22 (42 per cent) developed tumors. There was apparently no difference in the number of tumors induced by methylcholanthrene in the rats fed *p*-dimethylaminoazobenzene in addition, and in the controls. There was a certain difference in the distribution of the observed tumors between carcinomas and epitheliomas in the two groups. It seems difficult to decide whether this was due to the treatment of the one group with the carcinogenic dye or not.

The rats of series 73 received *p*-dimethylaminoazobenzene and urethane simultaneously with the diet. Eighteen per cent of the animals from this group surviving 9 months developed pulmonary adenomas. Of 38 controls that received the same amount of urethane without the dye, 26 per cent showed pulmonary adenomas. It is sometimes difficult to determine whether the microscopic lesions observed in the lungs of these animals are adenomas or not. Therefore, the numbers cannot be considered very accurate. But it can be said that the results obtained in the present experiments do not warrant the statement that the treatment with *p*-dimethylaminoazobenzene had any influence on the ability of urethane to produce pulmonary adenomas in rats.

The animals of series 70 received the same treatment of *p*-dimethylaminoazobenzene and methylcholanthrene as series 67 with the only difference that the carcinogenic diet had been prepared with cod liver oil instead of sesame oil. This modification was made because a previous study had shown that the application of cod liver oil reduces the number of tumors produced by methylcholanthrene in rats (4). It seemed of interest to produce a smaller number of local tumors in one group without reducing the dose of the carcinogen in order to check the correlation of the occurrence of hepatic and methylcholanthrene-induced tumors in the same animal as presented in Table II; a very high incidence of both types of tumor gives less accurate results in this calculation. The reduction of local tumors observed in this series compared with that of series 67

is similar to that previously described, namely 50 per cent. The number of liver tumors observed in this series was somewhat lower than that of the other series, but the difference cannot be considered to be significant.

TABLE II: COMPARISON OF EXPECTED AND FOUND PERCENTAGE OF RATS THAT DEVELOPED HEPATIC TUMORS AND OTHER TUMORS AFTER TREATMENT WITH *p*-DIMETHYLAMINOAZOBENZENE AND ANOTHER CARCINOGEN

Series no.	Animals with hepatic tumors, %	Animals with other tumors, %	Animals with hepatic and other tumors, found, %	Animals with hepatic and other tumors, expected, %
67	78	39	35	30
70	61	22	9	13
73	77	18	12	17

In series 68, which received *p*-dimethylaminoazobenzene only, 1 retroperitoneal polymorph cellular sarcoma was found; in series 73, which received the carcinogenic dye and urethane, 1 retroperitoneal sarcoma and 1 adenoma of the kidney cortex were observed. The sarcomas must be considered spontaneous. As the kidney adenoma was the first case observed among our animals, treated and untreated, it is impossible to decide whether it was spontaneous or elicited by the treatment. The 2 sarcomas found in 41 animals which survived 1½ years should be expected to develop without any treatment as the spontaneous tumor rate is about 5 per cent. Apparently there was no influence of the treatment on this spontaneous tumor rate.

In Table II the tumors observed in the series treated with two different carcinogens are summarized in respect to their simultaneous occurrence in one animal. Metastases have not been included. Only those rats which survived 9 months have been included. The mathematical probability for the simultaneous development of 2 types of tumor produced by 2 independent carcinogens in 1 animal has been calculated according to the formula:

$$P = \frac{a}{q} \cdot \frac{a}{v}$$

where a is the total number of animals, q the number of animals bearing one type of tumor and v the number of animals bearing the other type of tumors. These calculated values are compared in the table with the values found in each series. It can be seen from this comparison that the calculated and found values do not vary considerably. The number of animals included in the calculation is not great enough to reveal slight differences between the two values.

DISCUSSION

The experiments presented in this paper were performed in order to investigate the influence that may be exerted by one carcinogen on the action of another, when both carcinogens were applied simultaneously. The results obtained with our strain of albino rats do not justify the conclusion that interaction occurred. The incidence of liver tumors produced by feeding a diet containing *p*-dimethylaminoazobenzene did not vary significantly when the animals were treated at the same time with methylcholanthrene by injection, or when urethane was given simultaneously with the diet. This negative finding indicates that the carcinogens, methylcholanthrene and urethane, did not influence the production of hepatomas by the carcinogenic dye. Urethane alone produced a few hepatomas in rats when fed in the same amount without *p*-dimethylaminoazobenzene, but its action in producing liver tumors was very weak compared with that of the dye. The dose of the carcinogenic dye used in the present experiments was 25 per cent of that usually applied, but the application was continued for the whole experimental period of 15 months because it was hoped that by prolonging the experimental period it would be easier to observe a possible influence on the action of the second carcinogen applied.

It has been stated that a diet containing *p*-dimethylaminoazobenzene renders rats more susceptible to carcinogenic stimulation (7). It should be expected in this case, that tumor development with methylcholanthrene would be enhanced in rats receiving such a diet. No such stimulation was observed in the present experiments. The same was true for the animals receiving *p*-dimethylaminoazobenzene and urethane at the same time. In this case the number of pulmonary adenomas, which must be regarded as caused by urethane, did not differ significantly from that obtained in the controls that were treated with urethane only.

Similar results have been obtained by Rusch and his collaborators in experiments on the additive effect of ultraviolet light and carcinogenic hydrocarbons (8). Although mice rendered procarcinogenic with one hydrocarbon developed tumors with another, ultraviolet light did not have the same effect nor did it increase the carcinogenicity of Shope papilloma virus. A stimulation of the development of spontaneous pulmonary adenomas in mice injected subcutaneously with carcinogenic hydrocarbons has been observed by Andervont (1), while various authors found such substances to have an action on established spontaneous or implanted tumors, slowing their growth and even causing regression (9). Our results are no evidence of the existence of a similar influence of *p*-dimethylaminoazobenzene on tumors produced by methylcholanthrene or urethane and vice versa.

The comparison of the expected and found percentages of animals bearing two types of tumors elicited

by two differently acting carcinogens does not prove the existence of a linkage between the two actions. The values do not differ significantly. This result is the opposite of that obtained by Blum with inbred mice in which cutaneous tumors were induced by ultraviolet radiation and which have a high spontaneous rate for pulmonary tumors (2). A certain linkage between the appearance of both types of tumors in the animals has been found by means of an exact statistical analysis of the development time of pulmonary adenomas in animals bearing cutaneous tumors. The number of animals used in the present experiments is not sufficient for a similar analysis of the results. Dunlap and Warren observed a percentage of lung tumors in pure strain mice injected with carcinogenic hydrocarbons which was several times higher in the animals developing local tumors than in those which did not (3).

The failure to observe a correlation between the occurrence of two types of tumor gives rise to a number of theoretical questions. It has been assumed that the development of cancer in a given individual depends on the special genetical and environmental conditions. The genetical conditions may be such that they favor development of neoplastic growth or that they inhibit it. By selecting animals with such different genetical characteristics, the inbred strains with varying tumor incidence have been obtained. But, these strains do not show an equally high susceptibility or resistance toward all types of tumor. Susceptibility therefore cannot be dependent on a single genetical factor, but special factors must be assumed for the different kinds of tumors. Nevertheless, it has been proposed that one factor may exist in certain inbred strains which produces higher susceptibility for all types of tumors and that each special type depends moreover on other genetical factors. This should be reflected by a certain linkage of susceptibility toward different types of tumors. While the authors cited above observed such a linkage in their inbred strains of mice, it was not detectable in our experiments performed with an non-inbred strain of rats. Similar negative findings were obtained with non-inbred mice in which no linkage between methylcholanthrene-induced sarcomas and pulmonary adenomas could be observed (5).

It therefore seems likely that a factor or factors can exist in inbred strains which render the animals more susceptible to any kind of tumor, but that such a factor is not necessarily present in any given strain.

SUMMARY

Three groups of rats were fed a diet containing 0.015 per cent of *p*-dimethylaminoazobenzene and simultaneously were treated with methylcholanthrene by intraperitoneal injection or received in addition 0.15 per cent ethyl urethane in the diet. The incidence of hepatomas observed after 9 to 15 months were 61 to 78 per cent. The controls fed only the carcinogenic diet developed hepatomas in 75 per cent.

The incidence of sarcomas and epitheliomas in the groups injected with methylcholanthrene and fed the carcinogenic diet did not vary significantly from that observed in the controls, which were injected with the carcinogenic hydrocarbon only. The number of pulmonary adenomas observed in rats fed *p*-dimethylaminoazobenzene and urethane simultaneously did not vary to a significant degree from that obtained in the control group which was fed a diet containing urethane but no carcinogenic dye.

A comparison of the calculated and observed number of animals that had hepatic liver tumors and other tumors at the same time showed no significant difference.

There was apparently no mutual influence of two carcinogenic agents applied at the same time under the experimental conditions employed.

REFERENCES

1. Andervont, H. B., and Edwards, J. E. Hepatic Changes and Subcutaneous and Pulmonary Tumors Induced by Subcutaneous Injections of 3, 4, 5, 6-Dibenzcarbazole. J. Nat. Cancer Inst., **2**:139-149. 1941.
2. Blum, H. F. Relationships between Spontaneous Tumors of the Lung and Cutaneous Tumors Induced with Ultraviolet Radiation in Strain A Mice. J. Nat. Cancer Inst., **5**:89-97. 1944.
3. Dunlap, C. E., and Warren, S. The Incidence of Primary Lung Tumors in Mice with Induced Sarcomas. Cancer Research, **2**:685-687. 1942.
4. Jaffé, R., and Jaffé, W. El aceite de higado de bacalao en la tumefaccion artificial de la rata. Rev. Sudamer. Morfol., **3**:84-88. 1945.
5. Jaffé, W. G. Possible Linkage Between the Development of Local Tumors and Pulmonary Adenomas Induced by Methylcholanthrene in Non-Inbred Mice. Cancer Research, **7**:117-119. 1947.
6. Jaffé, W. G. Carcinogenic Action of Ethyl Urethane on Rats. With an Appendix by R. Jaffé. Cancer Research, **7**:107-112. 1947.
7. Nishiyama, Y. Experimentelle Erzeugung des Sarkoms bei Ratten durch wiederholte Injektionen von Glucoselösung. Gann, **32**:85-98. 1938.
8. Rusch, H. P., Kline, B. E., and Baumann, C. A. The Nonadditive Effect of Ultraviolet Light and Other Carcinogenic Procedures. Cancer Research, **2**:183-188. 1942.
9. Stern, K., and Willheim, R. The Biochemistry of Malignant Tumors. Brooklyn: Reference Press. 1943.

Possible Linkage Between the Development of Local Tumors and Pulmonary Adenomas Induced by Methylcholanthrene in Non-Inbred Mice

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(Received for publication August 9, 1946)

The present paper deals with an analysis of pulmonary adenomas observed in 550 mice injected with various doses of methylcholanthrene for the study of other problems. A review of the pulmonary tumors found in these animals showed a distribution which could not be expected, according to previous publications, by other authors who had worked with inbred mice (1, 2).

MATERIALS AND METHODS

The experiments were performed with our non-inbred strain of albino mice, which has a spontaneous tumor rate of about ½ per cent, pulmonary adenomas not included. These tumors are subcutaneous and retroperitoneal sarcomas, and very few mammary adenomas. The incidence of pulmonary adenomas was 5 per cent at the age of 4 months, 15 per cent at the age of 6 months and 25 per cent at the age of 1 year.

The animals were injected with a solution of methylcholanthrene in sesame oil or olive oil at the age of 2 months and kept on a stock diet. The series which received the highest dose showed a considerable mortality. Each animal that died spontaneously, or was killed after the end of the experiment, was autopsied and a gross examination for the existence of tumors performed. Only in doubtful cases was the material fixed for microscopic study. The lungs were put in Bouin's fixation liquid for one minute and then fixed in 10 per cent formol for 24 hours. This procedure gave best results to distinguish superficially located lung tumors which stain slight yellow and stand out over the surface, whereas pneumonic nodules remain white. The number of visible adenomas was recorded for each lung examined.

The animals developing local tumors were not killed except when the growth became so large that death was to be expected very soon. The animals were killed 3, 4, or 5 months after the injection.

RESULTS

In Table I, the percentages for the animals bearing local tumors and/or pulmonary adenomas are presented;

TABLE I: NUMBER OF MICE DEVELOPING LOCAL AND/OR LUNG TUMORS AFTER INJECTION WITH METHYLCHOLANTHRENE AND OF THOSE BEARING BOTH TYPES OF TUMORS AS CALCULATED AND AS FOUND

Treatment		Period after methylcholanthrene injection: 3 mos.	4 mos.	5 mos.
0.9 mgm. Methylcholanthrene intraperitoneally	Number	37	37	74
	% with pulmonary tumors	46	43	76
	% with local tumors	27	30	15
	% with both types of tumor expected	12	13	12
	% with both types of tumor found	13	19	13.5
0.9 mgm. Methylcholanthrene subcutaneously	Number	62	164	32
	% with pulmonary tumor	26	45	47
	% with local tumor	68	80	72
	% with both types of tumor expected	18	36	34
	% with both types of tumor found	19	37	28
0.45 mgm. Methylcholanthrene subcutaneously	Number		78	
	% with pulmonary tumors		32	
	% with local tumors		67	
	% with both types of tumor expected		21	
	% with both types of tumor found		22	
0.225 mgm. Methylcholanthrene subcutaneously	Number		68	
	% with pulmonary tumors		31	
	% with local tumors		41	
	% with both types of tumor expected		13	
	% with both types of tumor found		12	

in the last lines the expected and found percentages of animals bearing both local tumors and pulmonary adenomas are indicated. The expected numbers were calculated using the formula:

$$P = \frac{a}{q} \cdot \frac{a}{v}$$

where P is the mathematical probability that both types of tumors are found in the same animal, a is the total number of animals examined, q the total number of animals bearing local tumors and v the total number of animals bearing pulmonary adenomas. A comparison of the expected and observed values shows that although in 6 experiments the found value was higher than the expected, the quantitative difference was never significant.

As endocrine factors are of importance in the development of certain tumors, the results were analyzed according to sex. Table II shows that in males the linkage between the development of local and lung tumors is more pronounced than in females. But the quantitative difference of the calculated and found numbers of animals bearing both types of tumors are never large enough to be significant.

TABLE III: NUMBER OF ADENOMA NODULES IN LOCAL TUMOR-BEARING AND TUMOR-FREE MICE

Treatment		Period after injection of methylcholanthrene: 3 mos.	4 mos.	5 mos.
0.9 mgm. Methylcholanthrene intraperitoneally	With local tumors	3	10	16
	Without local tumors	6	10	17
0.9 mgm. Methylcholanthrene subcutaneously	With local tumors	3	4	9
	Without local tumors	3	3	4
0.45 mgm. Methylcholanthrene subcutaneously	With local tumors		1.7	
	Without local tumors		1.6	
0.225 mgm. Methylcholanthrene subcutaneously	With local tumors		2.4	
	Without local tumors		2.7	

DISCUSSION

The results presented do not justify the conclusion that there exists a linkage between the susceptibility for the development of local sarcomas elicited by methylcholanthrene and adenomas of the lungs in our strain of mice. The percentages of animals bearing both types of tumor never varied to a significant degree from those expected from calculation of the mathematical probability for the simultaneous development of both types of tumor. Moreover, the mean number of adenoma nodules of the animals bearing sarcomas was not greater than that of those animals which did not.

Other authors working with inbred strains of mice obtained results the opposite of those presented in this paper. Blum (1), analyzing the tumors observed in a great number of strain A mice with cutaneous tumors induced by ultraviolet radiation, found a reduction of

TABLE II: NUMBER OF MALE AND FEMALE MICE, EXPECTED AND FOUND, BEARING LOCAL AND PULMONARY TUMORS

Treatment		Period after methylcholanthrene injection: 3 months male	3 months female	4 months male	4 months female	5 months male	5 months female
0.9 mgm. Methylcholanthrene intraperitoneally	Number	19	18	22	15	42	32
Both types of tumor, %	Expected	10	15	20	27	8	17
	Found	16	22	27	7	10	19
0.9 mgm. Methylcholanthrene subcutaneously	Number	32	30	80	84	21	11
Both types of tumor, %	Expected	23	22	34	37	19	39
	Found	16	23	36	37	22	36
0.45 mgm. Methylcholanthrene subcutaneously	Number			49	25		
Both types of tumor, %	Expected			21	14		
	Found			25	12		
0.225 mgm. Methylcholanthrene subcutaneously	Number			36	28		
Both types of tumor, %	Expected			11	14		
	Found			14	11		

In Table III the results are analyzed according to the number of adenoma nodules found in animals bearing sarcomas and those that did not. In this case there was no significant difference.

The subcutaneous injection of the same dose of methylcholanthrene gave rise to more sarcomas and fewer pulmonary adenomas than the intraperitoneal application. The fact that the mice given methylcholanthrene intraperitoneally and surviving longest showed fewer sarcomas than those that died earlier is explainable because the sarcomas killed the mice quickly and the surviving animals were mostly those resisting the development of this kind of tumor.

pulmonary adenomas caused by the treatment; but his calculations proved the existence of a linkage between the development of local and pulmonary tumors, *e.g.* animals bearing local tumors had a higher susceptibility toward the development of pulmonary adenomas than those resisting the development of local tumors.

Dunlap and Warren (2) found that in pure strain Swiss mice injected with carcinogenic hydrocarbons the incidence of primary lung tumors was several times higher in those developing tumors at the site of injection than in those failing to develop local tumors.

Both experiments were performed with highly inbred mice. The authors conclude that a hereditary factor may exist which must affect the development of any tumor at any site in the body, while other factors may especially affect the growth of specific tumors. It is not possible from the data of the authors cited to decide whether the factor, active in stimulating any tumor growth, exists in each animal, or whether the mice strains are not homogeneous in this respect. It seems doubtful whether it will ever be possible to decide this question, as this factor is not the only one determining tumor development. From the results of our experiments it must be concluded that such an unspecific factor for tumor development is not necessarily detectable in any given strain, either because it is not present at all, or because its action may be so weak as hardly to manifest itself. This is in accordance with our results obtained in non-inbred rats, treated simultaneously with two different carcinogens, in which no linkage between the development of two types of tumors in the same animal was detectable (3).

SUMMARY

Non-inbred albino mice, 550 in number, were injected with various amounts of methylcholanthrene and studied with respect to the possible linkage between the development of local tumors and pulmonary adenomas in the same animal.

No significant linkage could be detected when the statistically expected and experimentally found numbers of mice bearing both kinds of tumors were compared. Neither was there a significant difference between the number of pulmonary adenoma nodules in animals bearing local tumors and those that did not.

When the linkage between the development of local and lung tumors was studied separately in males and females, this was found to be higher in males than in females, but the quantitative difference between the calculated and observed number of animals bearing local and lung tumors at the same time, never exceeded the average difference, *i.e.* was not significant.

It is concluded that an unspecific factor influencing any tumor development, which has been proposed to exist by other authors, is not necessarily detectable in any strain of mice.

REFERENCES

1. Blum, H. F. Relationships between Spontaneous Tumors of the Lung and Cutaneous Tumors Induced with Ultraviolet Radiation in Strain A Mice. J. Nat. Cancer Inst., **5**:89-97. 1944.
2. Dunlap, C. E., and Warren, S. The Incidence of Primary Lung Tumors in Mice with Induced Sarcomas. Cancer Research, **2**:685-687. 1942.
3. Jaffé, W. G. The Response of Rats to the Simultaneous Application of Two Different Carcinogenic Agents. Cancer Research, **7**:113-116. 1947.

Abstracts

Reports of Research

Activité comparée des trois principaux hydrocarbures synthétiques cancérigènes. [Study of the Comparative Carcinogenic Activity of the Three Most Important Synthetic Hydrocarbons.] Roussy, G., and Guérin, M., [Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:66-73. 1942.

The synthetic hydrocarbons studied were the 1, 2, 5, 6-dibenzanthracene, 3, 4-benzpyrene, and methylcholanthrene. Comparative studies already performed and published had given contradictory results in some instances. The authors experimented with mice and rats. Only the activity of weak doses was tested. The material was injected subcutaneously, using solutions of 1/1,000 or 1/10,000 in olive oil. The conclusions were that methylcholanthrene and benzpyrene have an almost equal activity in rats; methylcholanthrene is more active in mice; dibenzanthracene is the least active, both in mice and rats. The minimal active dose for these compounds was found to be in the neighborhood of a gamma. This finding compares favorably with the observations relating to the activity of certain hormones.—R. J.

Effets antagonistes de différentes benzacridines envers le pouvoir cancérigène du méthylcholanthrène. [Antagonistic Effects of Several Benzacridines toward the Carcinogenic Power of Methylcholanthrene.] Buu-Hoï, N.-P., Lacassagne, A., Lecoq, J., and Rudali, G. [Polytechnic Sch., Paris, France] *Compt. rend. Soc. de biol.*, **139**:485-486. 1945.

Previous work had established that the mixture of a polycyclic hydrocarbon of low carcinogenic activity with a hydrocarbon of similar molecular structure, but highly carcinogenic, diminishes the toxic effects of the latter and delays the onset of tumors. A series of new synthetic substances has been tried, whose molecular structure would suggest that they might inhibit the activity of the methylcholanthrene. These substances were different compounds of dimethyl-7,8-benzacridine, and dimethyl-5,6-benzacridine. The results showed that only the dimethyl-5,6-benzacridines had an antagonistic effect on the carcinogenic action of methylcholanthrene. It was observed further that derivatives of dimethyl-7,8-benzacridines, used alone, possessed a certain degree of carcinogenic activity.—R. J.

Activité cancérigène de certains dérivés méthylés des benzacridines angulaires. [Carcinogenic Activity of Certain Synthetic Methyl Benzacridines.] Lacassagne, A., Rudali, G., Buu-Hoï, N.-P., and Lecocq, J. [Polytechnic Sch., Paris, France] *Compt. rend. Soc. de biol.*, **139**:955-957. 1945.

Several varieties of synthetic methyl benzacridines were tested on 6 lots of mice of the strains 17 and 30. Applications to the neck were continued for several months. The results showed that only the 7,8-benzacridines were capable of provoking cancer. The 5,6-benzacridines had no carcinogenic activity. Among the 7,8-benzacridines, the 3,4-dimethyl-7,8-benzacridine was found to be as active as methylcholanthrene. The stability and the ease with which this synthetic compound is produced is interesting.—R. J.

D.B.E.: A New Synthetic Oestrogen. Greene, R. [Metropolitan Hosp., London, England] *Brit. M. J.*, 1:9-10. 1946.

The compound is α,α-di-(*p*-ethoxyphenyl)-β-phenyl bromoethylene. In ovariectomized mice it has an oral threshold similar to that of estradiol and much higher than that of stilbestrol. It differs from these compounds in that its action is much more prolonged. The long duration appears to depend upon storage in the fat of the body. It has been used with some success in the treatment of menopausal symptoms. The effects of this compound in 3 cases of carcinoma of the prostate are described in detail. The author concludes that D.B.E. is a substance of great theoretical interest, but in some respects it is inferior to the other synthetic estrogens. It is doubtful whether the advantage of weekly administration over administration 3 times a day is sufficient to warrant the general introduction of another of these biologically active agents.—E. L. K.

The Status of Hormonal Bioassay in Malignant Disease of the Testicle. A Review of the Literature. Francis, R. S. [R.N.V.R., England] *Brit. J. Surg.*, **33**:173-178. 1945.

An elaborate tabulation of the results of the Aschheim-Zondek test in over 300 cases recorded in the literature is presented. The results in mouse units per liter may be summarized as follows. (1) Cases of seminoma gave an average level well below 1,000; 75% were below 500 and only 25% were below 100. (2) Cases of chorinonepithelioma, adenocarcinoma, carcinoma, malignant teratomas of all types gave values of 1,000 to 50,000, and 1 to 3 million in extreme cases.—E. L. K.

Hormona tumoral gonadotrópica en los cancerosos. ["Tumoral" Gonadotropic Hormone in Cancer Patients.] Roffo, A. H. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:419-588. 1944.

A steroid "E," which acts as an estrogen in immature female rats and as an androgen in immature male rats, and which increases the volume of the spleen to 2 or 3 times normal size, has been found in cancerous tissues, blood, and urine of rats and human patients with malignant tumors. A reaction for the diagnosis of cancer, based on detection of this steroid, has proved its worth in 1,000 patients. Tests were positive in all instances of cancer, even in the presence of many early and cutaneous lesions.—M. H. P.

Chorionic Gonadotropin in the Diagnosis of Testicular Tumors. Brewer, J. I. [St. Luke's Hosp., Northwestern Univ. Sch. of Med., Chicago, Ill.] *Arch. Path.*, **41**:580-591. 1946.

Two cases are presented in which tests of the urine for chorionic gonadotropin indicated that chorionic tissue was present in the primary tumor. Such tissue was not identified in sections from various portions of the tumors removed at operation, but in both cases metatases developed which were found on postmortem examination to consist of choriocarcinoma.—J. G. K.

For information regarding microfilm copies of articles, abstracts of which appear in *Cancer Research*, application should be made to the Photoduplication Section, Army Medical Library, 7th Street and Independence Avenue, S.W., Washington 25, D. C.

Caractéres généraux des maladies à virus cancérigènes. Maladies bryocytiques ou à sporozoaires. [General Characteristics of Diseases Caused by Carcinogenic Viruses. Bryocytic or Sporozoan Diseases.] Bosc, F. J. [Montpellier, France] *Bull. Assoc. franç. p. l'étude du cancer*, **29**:94-104. 1940.

It is reported that attempts to treat epithelioma and breast carcinoma by means of so-called specific antisera produced against tumor or homologous normal tissue have failed.—A. C.

Action des radiations sur la transformation maligne du papillome infectieux du lapin. [Action of Radiation on the Malignant Transformation of Infectious Papilloma of Rabbits.] Lacassagne, A., and Rudali, G. *Bull. Assoc. franç. p. l'étude du cancer*, **30**:74-89. 1942.

Two series of experiments are reported, one in which x-rays were used, the other in which radon was the radioactive agent. In all animals, the Shope papilloma was inoculated in 2 separate areas of the body, one of these serving as a control, the other being irradiated. It was observed that the radiation has little effect on the behavior of the papilloma and its tendency to malignant transformation. The frequency of this transformation seems to be slightly increased after irradiation. When irradiation is given before the virus inoculation, no papilloma appears, yet other areas of the same animal are susceptible to the agent.—R. J.

Los estudios de Roffo sobre el colesterol en la génesis del cáncer. Su método para determinar el pre-cáncer en las lesiones hypercolesterínicas y fluorescentes de la piel. [Roffo's Studies on Cholesterol in the Genesis of Cancer. His Method for Determining a Precancerous State in Hypercholesterinic and Fluorescent Lesions of the Skin.] Fonso, F. S. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:629-684. 1944.

A review with 24 references, and an illustrated presentation of 16 cases of cutaneous cancer and precancerous lesions manifesting fluorescence revealed by the Wood lamp is given.—M. H. P.

The Metabolism of Chrysene in the Rat. Berenblum, I., and Schoental, R. [Univ. of Glasgow, Glasgow, Scotland] *Biochem. J.*, **39**:64. 1945.

When chrysene is injected interperitoneally in arachis oil, the 3-methoxy derivative appears in the feces. The 2-position is the most reactive in mono-substitution.—E. L. K.

Iron Content of the Serum in Lesions of the Liver and Bile Passages. Brøchner-Mortensen, K. [Kommune Hosp., and General Lab. of Nat. Health Insurance Physicians, Copenhagen, Denmark] *Acta med. Scandinav.*, **112**:277-290. 1942.

The iron content of the blood serum was found to be increased to over 200% in 18 of 26 patients with acute hepatitis, and in 1 of 5 with cirrhosis of the liver, but in only 1 of 12 with obstructive jaundice due to cancer, and in none of 7 with cholelithiasis. Application of the determination to supplementary differential diagnosis is suggested.—M. H. P.

A propósito de una dieta anticancerígena. [An Anticarcinogenic Diet.] Alem, C. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:335-336. 1944.

Because of Roffo's observations on the carcinogenic action of cholesterol derivatives, a diet excluding animal fats and restricting cholesterol containing foods is recommended for combating cancer. Vegetable oils, sufficient vitamins, and easily digested proteins are recommended, and the limitation of carbohydrates is suggested.—M. H. P.

On the Agglutination of the Blood Platelets under Normal and Pathological Conditions. Øllgaard, E. [Copenhagen County Hosp., Copenhagen, Denmark] *Acta med. Scandinav.*, **115**:1-21. 1943.

The agglutination of blood platelets from plasma that has stood at 42° C. for 3 hours and then treated with $HgCl_2$ has been found to be above normal in afebrile diseases which have increased sedimentation rates. These include cancer involving various organs and also lymphogranulomatosis. However, there was no parallelism between agglutination and sedimentation rate.—M. H. P.

Les leucoses à forme tumorale. Contribution à l'origine des lymphosarcomes. [Leukemia. Discussion on the Origin of Lymphosarcomas.] Mallet, L., and Gasne, L. [Tenon Hosp., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:178-188. 1943.

After a review of the experimental work performed mainly on mice and to a lesser extent on rats, the clinical and hematological aspects of leukemia in human pathology are studied. The authors do not believe in the virus origin of leukemia, since it is impossible to transmit the disease in animals without the use of living cells. As is known, leukemias are provoked by many physical or chemical agents. The possibility of mutation-provoking factors is discussed.—R. J.

Studies in Hodgkin's Syndrome. III. The Relationship of Tubercle Bacilli to Hodgkin's Syndrome. Hoster, H. A., Doan, C. A., and Schumacher, M. [Ohio State Univ., Columbus, Ohio] *J. Lab. & Clin. Med.*, **30**:675-677. 1945.

A series of chickens, guinea pigs, and rabbits were inoculated with fresh macerated lymph node and splenic tissue emulsions obtained surgically from patients with Hodgkin's syndrome and other diseases of widely differing etiologies. In each case carefully chosen media were inoculated with tissue emulsion. In no instance were tubercle bacilli found except in the case of histologically, bacteriologically, and clinically proved tuberculosis.—Author's summary.—J. G. K.

Épithélioma mammaire transplantable developpé sur un adénofibrome spontané du rat. [Transplantable Mammary Epithelioma Developing in a Spontaneous Adenofibroma in a Rat.] Roussy, G., Guérin, P., and Guérin, M. [Cancer Inst., Univ. of Paris, Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:150-159. 1943.

The mammary tumors in rats are most frequently adenofibromas. Their sarcomatous transformation is relatively frequent, but their transformation into glandular epitheliomas is exceptional. In the case described, such a transformation was observed. Successive transplants to other rats showed a progressive modification of the histological picture toward the fusiform sarcomatoid type. The possibility of a metamorphosis of epitheliomatous cells to a sarcomatoid type cell is suggested.—R. J.

Premiers résultats concernant la transplantation chez le Rat de tissu en voie de cancérisation. [Preliminary Results concerning the Transplanting in Rats of Tissue in the Process of Becoming Cancerous.] Roussy, G., Guérin, M., and Guérin, P. [Cancer Inst., Univ. of Paris, Paris, France] *Compt. rend. Soc. de biol.*, **137**:759-760. 1943.

This technic of transplantation was used in the study of the mechanism of action of chemical carcinogenesis, and also to study the behavior of tissue grafts in the process of becoming cancerous. Preliminary results seem to indicate that (1) autotransplants of such tissues have

more chances to develop cancer than homografts, (2) the development of a tumor does not depend on the general dissemination in the organism of the carcinogenic substance, but is due to the local action of this substance.—R. J.

Cultural Characteristics of a Hemangioendothelioma. MURRAY, M. R., and STOUT, A. P. [Coll. of Physicians and Surgeons, Columbia Univ., and Presbyterian Hosp., New York, N. Y.] *Am. J. Path.*, **20**:277-283. 1944.

A hemangioendothelioma of unusual morphological appearance is described and its behavior in tissue culture is discussed. This particular neoplasm is regarded as manifesting itself in the form of a primitive or somewhat dedifferentiated vascular endothelium.—J. G. K.

A Simple Technique for Preparing Vaginal Smears. RUBENSTEIN, B. B., and GUTERMAN, H. S. [Michael Reese Hosp., Chicago, Ill.] *Am. J. Obst. & Gynec.*, **50**:565-567. 1945.

A simple technic for the preparation of vaginal smears is reported. It is so simple that patients themselves can make the preparations.—A. K.

Sur la présence de vésicules ou boutons embryonnaires géants dans les métastases ganglionnaires des embryomes testiculaires. [Presence of Vesicles or Giant Embryonic Buds in Ganglion Metastases of Testicular Embryomas.] SABRAZÈS, J., and PEYRON, A. [Anticancer Center, Bordeaux, and Pasteur Inst., Paris, France] *Compt. rend. Soc. de biol.* **135**:350-353. 1941.

A histological study of clinical material.—G. H. H.

Studies on Tumors of the Testis. II. The Morphology of Testicular Tumors of Dogs. HUGGINS, C., and PAZOS, R. [Univ. of Chicago, Chicago, Ill.] *Am. J. Path.*, **21**:299-306. 1945.

Evidence is presented that interstitial cell tumors are derived from Leydig cells, tubular adenomas from Sertoli cells, and seminomas from germinal epithelium.—J. G. K.

Gráficos para la determinación de la calidad de un haz de rayos en base a la capa hemirreductora. [Graphs for Determining the Quality of a Beam of Rays Based on the Half-Value Layer.] ROFFO, A. E., JR. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:149-162. 1944.

These graphs are based on the values of the mass absorption coefficients and the classic elementary absorption formula. The ordinates represent the half-value layer, experimentally determined for copper or aluminum, and the abscissas, corresponding to the points where the ordinates meet the curves, indicate the effective wave length of the beam of x-rays. Two other scales are placed beneath the graphs, one indicating the kv. corresponding to wave lengths, and the other the maximum kv. corresponding to the effective wave lengths.—M. H. P.

El uso de las sulfamidas en el cáncer. [The Use of Sulfonamides in Cancer.] ALEM, C. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:331-333. 1944.

Sulfonamides, administered locally, orally, intravenously, or intramuscularly, proved useful in controlling infection of cancerous areas, especially as an adjuvant to surgery or radiation. Sulfathiazole given orally was preferred.—M. H. P.

La defensa de la piel para las radiaciones actínicas solares. Profilaxis del cáncer cutáneo. Filtraje de los ultravioletas largos. Estudio de la clorófila. Su aplicación práctica in las pieles fotosensibles. [Protection of the Skin against Actinic Rays of the Sun. Prophylaxis of Cutaneous Cancer. Filtration of the Long Ultraviolet Rays. Study of Chlorophyll. Its Practical Application in Cases of Photosensitive Skin.] ROFFO, A. E., JR. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:589-611. 1944.

Two methods are recommended for prevention of cancer of the skin: (1) pigmenting the skin physiologically by sunbaths graduated in accordance with cutaneous sensitivity, measured previously; (2) protecting the skin with pomades, pastes, or oils containing a substance, preferably chlorophyll, that absorbs the long ultraviolet rays of sunlight.—M. H. P.

La reacción Roffo. Su importancia como medio de diagnóstico precoz de las neoplasias. Sus modificaciones en el curso de los tratamientos aplicados en cancerologia. Su valor pronóstico. [Roffo's Reaction. Its Importance as a Means of Early Diagnosis in Neoplasm. Its Changes in the Course of Cancer Therapy. Its Prognostic Value.] MOGUILEVSKY, L. [Inst. of Exper Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:621-627. 1944.

Tests of Roffo's neutral red reaction in 30,961 instances have given correct positive results in 96% of patients with cancer, and false positive results in 16% of patients without cancer. The reaction is positive from very early stages of the disease, becomes negative within 1 month after complete surgical extirpation of the growth, and becomes negative more slowly after radiotherapy. When the Roffo reaction does not change after treatment, this indicates that the treatment has not modified the course of the disease.—M. H. P.

Cancer Research and Benefit to Patients. HAMMETT, F. S. [Lankenau Hosp. Research Inst., North Truro, Cape Cod, Mass.] *Science*, **103**:714. 1946.

A plea for cooperative investigation in cancer research for the purpose of exploring fully existing leads which contain promise of something of practical benefit to the cancer patient.—R. B.

Clinical and Pathological Reports

Clinical investigations are sometimes included under Reports of Research

DIAGNOSIS

El diagnóstico biológico del cáncer. [The Biologic Diagnosis of Cancer.] CARRATALA, A. T. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:183-213. 1944.

The diagnostic value of Roffo's neutral red and splenic hyperplasia reactions has been confirmed by clinical tests. The neutral red reaction, performed on the blood serum *in vitro*, gave over 90% correct positive results in several thousand patients with cancer of the alimentary tract, oral cavity, skin, urogenital organs, breast, lung, mediastinum, thyroid, parathyroid, or bone, or with leukemia, lymphosarcoma, or lymphogranuloma. The splenic hyperplasia reaction, observed in rats injected with blood serum from cancer patients, gave results for 25 patients with

neoplastic disease that differed markedly from the results for 25 patients with other illnesses; in the former group, the weight of the spleen of the test rats on the fourth day after injection ranged from 1/70 to 1/185 of the body weight, while in the latter group spleen weights varied from 1/185 to 1/400 of the body weight. Blood from pregnant women also produced splenic hyperplasia, but this is not regarded as a serious drawback to the use of the reaction for cancer diagnosis. Literature on chemical and biological tests for cancer is reviewed, and 45 references are given.—M. H. P.

La eritrosedimentación en gota gruesa como método de diagnóstico en el cáncer. [Erythrocyte Sedimentation in Thick Drops as a Method of Diagnosis in Cancer.] Luchetta, B. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:301-315. 1944.

The Bolen test (*J. Lab. & Clin. Med.*, **27**:1522. 1942) gave correct positive result in 66.21% of 79 patients with cancer of various organs, and correct negative results in 91.66% of 108 patients without cancer.—M. H. P.

A Tentative Test For The Diagnosis Of Pheochromoctyoma. Roth, G. M., and Kvale, W. F. [Rochester, Minn.] *J. Lab. & Clin. Med.*, **30**:366-368. 1945.

Preliminary report on the use of intravenous injections of small quantities of histamine base as a means of diagnosis is given.—J. G. K.

Roentogenological Manifestation of Malignancy of the Colon. Whitehead, L. J. [Richmond, Va.] *South. M. J.*, **38**:85-88. 1945.

The accuracy of diagnosis of carcinoma of the colon is estimated to be about 90% by some radiologists. The importance of knowing the results of proctologic and rectal examinations before the roentgen examination is undertaken, is stressed.—W. A. B.

Therapy—General

A Discussion of Angiomata and Pigmented Nevi. Bivings, L. [Atlanta, Ga.] *South. M. J.*, **38**:241-244. 1945.

From the standpoint of pediatrics, conservative treatment, especially of the hypertrophic type of angioma, seems indicated, since these frequently regress spontaneously. Surgery is recommended for the cavernous type, and CO_2 snow for flat angiomas.—W. A. B.

The Value of Radium in Various Diseases. Einhorn, M. [New York, N. Y.] *Med. Rec.*, **159**:98-100. 1946.

Case report of successful radium treatment of epithelioma of the left lower eyelid using a specially devised heavy metal cup with rubber radium container. Sixteen of 19 patients with carcinoma of the esophagus with obstruction were markedly improved after application of radium. Results of treatment of carcinoma of the stomach are likewise gratifying, especially with the bleeding type of malignant gastric lesion, by daily radium treatment using the rubber capsule technic. Obstructing pyloric cancer is similarly relieved. Cancer of the rectum was benefited in 1 instance with radium. The radiodiaphone was used in the treatment of all these cases. This instrument and others used for the radium treatement of cancer, are described.—V. J. L.

Dose Control in Radiotherapy. Jolles, B. [Gen. Hosp., Northampton, England] *Nature, London*, **157**:552. 1946.

In the treatment of malignant tumors the maintenance of an efficient connective tissue is as important as the delivery of a certain dose of radiation. The volume of tissue implanted with radium should be considered as an integral part of the living body and not in an abstract way.—M. L.

Skin and Subcutaneous Tissue

Le curage ganglionnaire systematique dans le traitement des adénopathies du cancer de la lèvre supérieure. [Systematic Removal of Lymph Nodes in the Treatment of Cancer of the Upper Lip.] Barbier, M., and Delarue, J. [Cancer Inst., Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:3-10. 1943.

The absence of enlarged regional lymph nodes does not exclude the possibility of extension or metastasis. When the lymph nodes are palpable, it is difficult to differentiate between an inflammatory and neoplastic process. For these reasons the authors suggest the systematic removal of the regional lymph nodes at the time of the treatment of the lip cancer. Frequently most of the lymph nodes of the neck have to be removed. A total of 53 patients treated in the manner prescribed by the authors have been followed since February 1934, and 13 cases followed for more than 5 years. Among these latter 6 did not have any recurrence of the disease.—R. J.

Cáncer de labio. [Cancer of the Lip.] Cazap, S. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:215-288. 1944.

A review with 108 references, and a summary of clinical statistics relating to 1,066 cases of cancer of the lip, 137 of leukoplakia, and 118 of precancerous lesions are given. Among the 1,321 patients, 93.64% were males, 6.36% females, and 52.32% were 40 to 60 years old. Cancer of the upper lip, comprising 5.30% of the lip cancer cases, affected the skin (rather than the mucosa) almost exclusively, and had approximately the same incidence in women as in men, apparently being unrelated to the use of tobacco. On the other hand, cancer of the lower lip, comprising 94.70% of the cases, affected the mucosa or semimucosa almost exclusively, and occurred in males to the extent of 96%, and seemed closely associated with the use of tobacco. Adenopathy occurred in 44.44% of the patients with lip cancer, 12.71% of those with precancerous lesions, and 8.09% of those with leukoplakia. Roffo's neutral red reaction gave positive or doubtfully positive results in 80.68, 25.42, and 18.98% of the patients with cancer, precancerous lesions, and leukoplakia, respectively. Therapy with electrocoagulation, x-ray, radium or combinations of these measures gave cures in 30.39% of the patients with lip cancer, 65.25% of those with precancerous lesions, and 78.10% of those with leukoplakia. For 5-year cures, the authors refer to an earlier series of 2,500 patients with cancer of the lip treated between 1923 and 1937, 19.3% of whom are alive and well at the time of writing.—M. H. P.

The Pilonidal Sinus. Sacrococcygeal Cyst Teratoma. Theis, F. V., and Rusher, M. W. [U. S. Naval Hosp., St. Albans, L. I., N. Y., and Presbyterian Hosp., Chicago, Ill.] *Surg., Gynec. & Obst.*, **79**:482-489. 1944.

Clinical discussion.—J. G. K.

Nervous System

Metastatic Tumors of Brain. Tom, M. I. [Univ. of Toronto, Toronto, Canada] *Canad. M. A. J.*, **54**:265-268. 1946.

This is a review of 82 cases of metastatic tumor in the brain. Metastases confined to the skull or dura mater and metastatic tumors causing compression of the spinal cord or spinal nerve roots have been excluded. The

majority of the tumors occurred in patients between the ages of 30 and 70, the sixth decade containing the largest number. About 60% of the tumors were in males. The most common primary site was the lung and the large preponderance of males in this group is noteworthy. The breast came second in frequency, all in women, in whom the primary was almost invariably recognized before symptoms of cerebral metastasis were observed. Multiple metastases occurred in 41 cases and in 41 only single metastases were found. The cerebral hemispheres, cerebellum, pituitary and subarachnoid space were the common metastatic sites. Complete autopsies were performed in 52 cases. Either primary or metastatic tumors of the lung were disclosed in 78.9%. This emphasizes the value of a complete physical and x-ray examination of the chest in the clinical differentiation of primary from secondary tumors of the brain.—M.E. H.

Intrasellar Meningioma and Multiple Cerebral Glioblastomas. KIRSCHBAUM, W. R. [Manteno, Ill.] *J. Neuropath. & Exper. Neurol.*, **4**:370-378. 1945.

A case of multiple subependymal glioblastoma multiforme is described. An intrasellar meningioma was also present in connection with the pituitary body. The author presents various postulations as to the probable origins of the tumors.—A. C.

Lipoma of the Corpus Callosum. A Clinicopathologic Study. LIST, C. F., HOLT, J. F., and EVERETT, M. [Univ. of Michigan, Ann Arbor, Mich.] *Am. J. Roentgenol.*, **55**:125-134. 1946.

The literature on lipoma of the corpus callosum is surveyed and two case reports are added. It is impossible to diagnose the condition from clinical evidence alone. Roentgenographic signs consist in increased radiolucency of the tumor, calcification, demonstration of an expanding mass in the anterior part of the corpus callosum, and agenesis of the posterior part of this structure. Surgical treatment is contraindicated.—E. H. Q.

Colloid (Paraphisial) Cysts of the Third Ventricle. WILSON, A. A. [Neurological Service, Charleston Gen. Hosp., Charleston, W. Va.] *West Virginia M. J.*, **42**:49-53. 1946.

A case report is presented of a rare, third ventricle tumor which was removed with excellent results. The pathological characteristics of these "colloid cysts" prevent their confusion with other cerebral neoplasms. They are diagnosed and localized by ventriculographic study and, since they are perfectly benign, their successful removal results in a permanent cure.—V. J. L.

Subdural Hydroma. GRANT, W. T. [Coll. of Med. Evangelists, Los Angeles, Calif.] *California & West. Med.*, **64**:246-249. 1946.

The primary description of the now well-defined clinical entity, subdural hydroma, has been reviewed by many authors. Such a lesion may be suspected with post-traumatic intracranial disturbance demonstrated by increased cerebrospinal fluid pressure, improvement following spinal puncture and encephalographic study. The symptoms are protean and inconstant following injury, but resemble those present in subdural hematoma. Fifty-one cases occurring in the past 7 years are cited and reviewed; the incidence of this lesion following depressed skull fracture of the "egg-shell" variety is high. Improvement is usually immediate when the hydroma is completely drained. Four cases are reported briefly to illustrate special points of interest.—V. J. L.

EYE

Classification and Pathology of Tumors of the Retina. CAMP, W. E. [Minneapolis, Minn.] *Minnesota Med.*, **28**:317, 318, 320. 1945.

A general consideration dealing particularly with the pathology and histogenesis of primary tumors of the retina is given.—R. A. H.

EAR

Malignant Growths of the Mastoid Process and Middle Ear. FIGI, F. A., and HEMPSTEAD, B. E. [Rochester, Minn.] *Minnesota Med.*, **28**:38-43. 1945.

A review of 38 cases seen at the Mayo Clinic during the 20-year period ending December, 1941, giving symptoms, clinical findings, treatment and course of the disease is presented. The authors conclude that malignant tumors involving this region are more frequent and more amenable to treatment than generally believed. They consider combined electrocoagulation, radical mastoidectomy and radiation as the treatment of choice.—R. A. H.

BREAST

Les grands syndromes douloureux d'envahissement osseux dans le cancer du sein. [The Painful Syndrome of Osseous Metastases from Cancer of the Breast.] DUCUING, J. [Toulouse, France] *Bull. Assoc. franç. p. l'étude du cancer*, **29**:36-58. 1940.

A discussion is given, without bibliography, concerning the frequency of osseous metastases from cancer of the breast, the time of appearance and characteristics of these metastatic lesions, the anatomical and clinical types of breast cancer that spread to bone, and the various clinical syndromes. Among 300 cases of breast cancer, 36 showed generalized cancer, and in 26 of these the bones were involved. A consideration of diagnosis of these lesions and their treatment, the relation of hyperparathyroidism and hematologic disturbances to diffuse cancer of bone, and the mechanism of invasion of bone are also given.—G. H. H.

The Effects of Orchiectomy on Primary and Metastatic Carcinoma of the Breast. TREVES, N., ABELS, J. C., WOODWARD, H. Q., and FARROW, J. H. [Memorial Hosp., New York, N. Y.] *Surg., Gynec. & Obst.*, **79**:589-605. 1944.

Detailed reports of 6 cases, with discussion is given. The authors conclude that bilateral orchiectomy may have been responsible for the temporary regression of the primary lesion in 2 instances. Metabolic observations revealed no changes in fluid and electrolyte balance, nitrogen balance and serum protein fabrication, urinary excretion of creatine, and the utilization of carbohydrate 12 to 18 days after operation. There was a marked lack of uniformity to endocrine treatment in the response of the blood changes caused by bone metastases.—J. G. K.

Ensayo de cirugía plástica en la extirpación del cáncer de la glándula mamaria. [Evaluation of Plastic Surgery in Extirpation of Cancer of the Mammary Gland.] ROFFO, A. E., JR. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:163-179. 1944.

Various types of incisions are described, with diagrams, and the author's experiences in a few cases are summarized without conclusions. A photograph shows plastic reconstruction in a patient whose breast was amputated without axillary evacuation; results are good in this type of case. Hindrances to plastic procedures are: (1) absence of skin, (2) imperfect circulation to the fleshy tissues left, as a consequence of the dissection and ligature of the blood vessels, and (3) previous radiation therapy.—M. H. P.

Tumors of the Breast. BELL, E. T. [St. Paul, Minn.] *Minnesota Med.*, **28**:560-564. 1945.

A general consideration of the occurrence, symptoms, pathology and treatment of breast tumors is presented.—R. A. H.

Female Genital Tract

Primary Masculinizing Tumors of the Ovary. Burket, J. A., and Abell, I. [Louisville, Kentucky] *Surg., Gynec. & Obst.*, **79**:651-654. 1944.

Report of a case is given in which the patient died on the 11th post-operative day. Permission for autopsy was refused. The arrhenoblastoma and the adrenal cell tumor of the ovary are discussed.—J. G. K.

The Curability of Granulosa-Cell Tumors. Jones, G. E. S., and Te Linde, R. W. [Johns Hopkins Hosp. and Univ., Baltimore, Md.] *Am. J. Obst. & Gynec.*, **50**:691-700. 1945.

Three cases of granulosa cell tumors of the ovary are reported in which recurrences developed not less than 14 years after the original operation. The 3 patients died 18, 20 and 21 years respectively after the removal of the primary tumor, in spite of the fact that the growths were well encapsulated and showed no evidence of metastates or implantation at the time of the first operation. One case recurred in spite of a bilateral salpingo-oophorectomy and a hysterectomy. One case with widespread inoperable abdominal metastases responded well to deep x-ray therapy given over a period of 3 years, but subsequent recurring tumors proved refractory to treatment. Total urinary estrogen values were within the range of normal found in premenopausal women. The values were, however, above those for postmenopausal individuals, the group in which these present patients fall. All 3 cases exhibited clinical signs of estrogenic activity on recurrence of the tumor.—A. K.

Granulosa Cell Tumor of the Ovary. A Clinical and Pathologic Review of Sixty-two Cases. Hodgson, J. E., Dockerty, M. B., and Mussey, R. D. [Mayo Foundation, and Mayo Clin., Rochester, Minn.] *Surg., Gynec. & Obst.*, **81**:631-642. 1945.

A detailed analysis and discussion.—J. G. K.

Teratoma of the Ovary. Curtis, A. H. [Northwestern Univ. Med. Sch., Chicago, Ill.] *Surg., Gynec. & Obst.*, **81**:504-506. 1945.

A case report, with discussion.—J. G. K.

Epidermoid Carcinoma Arising in an Endometrial Cyst of the Ovary. McCullough, K., Froats, E. R., and Falk, H. C. [St. John's Riverside Hosp., Yonkers, N. Y.] *Arch. Path.*, **41**:335-337. 1946.

A case report.—J. G. K.

Répartition topographique et délai d'apparition des Métastases extra-pelviennes dans le cancer de l'utérus. [Topographic Distribution and Time of Appearance of Extra-Pelvic Metastases in Cancer of the Uterus.] Gricouroff, G. [Inst. of Radium, Curie Foundation, Paris France] *Bull. Assoc. franç. p. l'étude du cancer*, **30**:90-117. 1942.

A statistical study concerning the location and time of extra-pelvic metastases in uterine cancer extending over a 19-year period (1919 to 1938) has led to the following conclusions: (1) Most of the metastases appear before the end of the third year. (2) There is no relation between the time at which the diagnosis is made and the treatment performed, and the frequency of metastases. A cancer treated early has almost the same likelihood of giving extra-pelvic metastases as an advanced case. (3) There is no evidence of increase of metastases following treatment of cancers, either by surgery of radium therapy, in comparison with untreated cases. (4) The topography of the primary cancer and its histological form have no prognostic value concerning the eventuality of metastases. (5) The abdominal metastases are the most frequent (periaortic adenopathies) among all localizations. (6) Some metastases have been successfully treated by surgery or radium therapy.—R. J.

The Management of Uterine Myomas. A Study Based on 1000 Consecutive Personal Cases and Illustrating the Technique of Panhysterectomy. Phaneuf, L. E. [Tufts Coll. Med. Sch., and Carney Hosp., Boston, Mass.] *Surg., Gynec. & Obst.*, **79**:182-191. 1944.

Clinical discussion.—J. G. K.

Le Cancer du col restant. A propos de 9 cas nouveaux observés à la clinique Curie. [Cancer of the Residual Cervix. A Discussion of 9 New Cases Observed at the Curie Clinic.] Lamarque, P., and Betoulières, P. [Cancer Regional Center, Montpellier, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:195-199. 1943.

Nine cases of cancer of the cervix after subtotal hysterectomy were observed from 1927 to 1937 at the Curie Clinic. The question of the predisposing effect of the hysterectomy is discussed. Concerning the therapeutic procedure, the main point is to detect the possible existence of a malignant process coexisting with a benign lesion which may conceal it. Hysterography, biopsy, and other appropriate diagnostic procedures should be done systematically before deciding on a subtotal hysterectomy. —R. J.

Unusual Cause of Death in Carcinoma of Cervix. Elwood, J. S. [Lurgan Hosp., Ireland] *Brit. M. J.*, **1**:82. 1945.

Forty-eight days after a Wertheim hysterectomy the patient developed intestinal obstruction due to a hard annular stricture just above the ileocecal valve. The muscular coats of the intestines were infiltrated with squamous-celled carcinoma.—E. L. K.

A Review of the Problem of Cancer of the Cervix Since the Use of Radium in 1912. Anspach, B. M. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Am. J. Obst. & Gynec.*, **50**:681-690. 1945.

The history of treatment of cancer of the uterine cervix is given. Diagnosis, technic of irradiation, technic of operation, results of different procedures, fallacies of cancer statistics, and prospects for the future are all carefully considered and evaluated. In summary, it is pointed out that a greater number of patients are presenting themselves to the physician in the early stages of the disease, and that irradiation therapy is becoming more widely available and effectively used. It is noted that since there has been a reduction in the risk of operative procedures, surgical results will continue to improve. Transfusion technics, bacteriostatic agents, nasal suction, improved methods of anesthesia are factors in increasing the chances of the patient for survival in the radical operative procedures. It is thought that perhaps a combination of irradiation therapy and surgery will give ultimately the best results.—A. K.

Pelvic Tumors. Pride, W. T. [Memphis, Tenn.] *South. M. J.*, **38**:539-541. 1945.

In one year (1943) the number of patients with gynecologic complaints receiving surgery at the John Gaston Hospital was 696. Of these, 548 were colored and 148 white; there were 237 fibromyomas, in the ratio of 3:1, with predominance among the colored patients. Of a total of 67 ovarian cysts, fewer occurred in colored patients than in white. The figures for carcinoma of the uterus and chorionepithelioma are not given in this series, but these tumors are discussed.—M. H. P.

Leucoplasia y kraurosis de la vulva. [Leukoplakia and Kraurosis of the Vulva.] Locatelli, V. H. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:715-730. 1944.

Three cases of leukoplakia without cancerous invasion were treated by total vulvectomy. Roffo's reaction was negative in each instance. Attention is drawn to the importance of leukoplakia as a forerunner of cancer of the vulva.—M. H. P.

Male Genital Tract

Teratoid Tumour and Carcinoma of the Testis. Wilson, F. H. [Montreal General Hosp., Montreal, Canada] *Canad. M. A. J.*, **54**:164-167. 1946.

Case report. The malignant tumor and metastases were mostly of an undifferentiated cell type and believed by the author to be an undifferentiated adenocarcinoma all of an endodermal origin in either the intestinal or respiratory epithelium.—M. E. H.

Cáncer del prepucio. Cirugia conservadora. Importancia de signos clinicos miccionales y del debridamiento dorsal. Nuestra experiencia y resultados. [Cancer of the Prepuce. Conservative Surgery. Importance of the Clinical Urinary Signs and of Dorsal Debridement.] Lacapraro, G., and Lacapraro, R. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:699-713. 1944.

Simple phimosectomy, performed with the electrical bistoury, is recommended for cancer shown by dorsal debridement and biopsy to be confined to the internal surface of the prepuce. Diagrams.—M. H. P.

Urinary System

Multicentric Bilateral Carcinoma of the Kidneys. Lisa, J. R. [City Hosp., Welfare Island, New York, N. Y.] *Am. J. Path.*, **21**:383-385. 1945.

A case report.—J. G. K.

Leiomyosarcoma Involving the Right Ureter. Rossien, A. N. [Rockaway Beach Hosp., and Triboro Hosp., New York, N. Y.] *Arch. Path.*, **41**:655-660. 1946.

Report of a case.—J. G. K.

Cystectomy for Carcinoma of the Bladder. Sweetser, T. H. [Minneapolis, Minn.] *Minnesota Med.*, **28**:987-992. 1945.

The surgical difficulties associated with extirpation of the bladder, as well as certain discouraging features of neoplastic diseases of this organ, are considered. Admitting serious limitations of the procedure, the author advocates cystectomy in selected cases and presents the case histories of 5 patients so treated.—R. A. H.

Adenocarcinoma of the Urachus Involving the Urinary Bladder. Rappoport, A. E., and Nixon, C. E. [Med. Corps, A. U. S.] *Arch. Path.*, **41**:388-397. 1946.

Report of a case.—J. G. K.

Oral Cavity and Upper Respiratory Tract

Les tumeurs malignes du rhinopharynx. Résultats éloignés de leur traitement par les radiation. [Malignant Tumors of the Rhinopharynx. Subsequent Results of their Treatment by Radiation.] Barclesse, F., and Dulac, G. [Curie Foundation, Paris, France] *Bull. Assoc. franç. p. l'étude du cancer*, **31**:160-177. 1943.

One hundred and three tumors were treated at the Curie Foundation. The authors emphasize the importance of radiographic exploration. Tumors extending to the skull were almost always beyond the resources of therapy. The treatment of localized cancers without adenopathy gave good results of long standing. A discussion follows on the technic which has to be used when applying Curietherapy to such cancers.—R. J.

El cáncer de la laringe en el Instituto de medicina experimental durante los ultimos cuatro años. [Cancer of the Larynx at the Institute of Experimental Medicine during the Last Four Years.] Jaeggli, O. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:319-327. 1944.

Among 407 histologically proved cases of cancer of the larynx, 99% were in males, 65% were in patients 40 to 60 years old, 77.42% gave a positive Roffo reaction, and 98% were in smokers. Radiotherapy gave poor therapeutic results. Of the 70 patients subjected to laryngectomy, with or without radiation, 57% were discharged as cured or have subsequently been found to be in good health.—M. H. P.

Sobre laringectomía. El cierre per priman de la herida operatoria. [Laryngectomy. Closing of the Surgical Wound by First Intention.] Ferrari, R. C. [Inst. of Exper. Med., Buenos Aires, Argentina] *Bol. Inst. de med. exper. para el estud. y trat. d. cáncer*, **21**:293-300. 1944.

Technic employed by the author since 1939 for therapy of cancer of the larynx by laryngectomy are described.—M. H. P.

Malignancies of the Larynx. Kernan, J. D. [New York, N. Y.] *Med. Rec.*, **159**:351-352. 1946.

Radiotherapy, at its inception, was reserved for the very advanced cases of carcinoma of the larynx, while surgery was employed in the treatment of early cases; the situation is now reversed. In intrinsic laryngeal carcinoma the prognosis is dependent upon the mobility of the cord and the location of the growth (best results with centrally located growths), and not upon the histological character of the tumor. Extrinsic laryngeal carcinoma carries a much worse prognosis whether treated surgically or by x-ray; though the latter is more effective with undifferentiated, and the former with differentiated, malignancies.—V. J. L.

Salivary Glands

Papillary Cystadenoma Lymphomatosum (Warthin's Tumor) of the Parotid Salivary Gland. Martin, H., and Ehrlich, H. E. [Memorial Hosp., New York, N. Y.] *Surg., Gynec. & Obst.*, **79**:611-623. 1944.

Report of 22 cases, with discussion.—J. G. K.

Intrathoracic Tumors

"Alveolar Cell Tumor" of The Lung. Further Evidence of Its Bronchiolar Origin. Herbut, P. A. [Jefferson Med. Coll. Hosp., Philadelphia, Pa.] *Arch. Path.*, **41**:175-184. 1946.

A description is presented of a very early "alveolar cell tumor" arising in a bronchiole and encountered as an accidental finding in an autopsy, and of a primary adenocarcinoma of the gall gladder with the classic gross and histologic alveolar cell distribution in the lungs. Furthermore an analysis of 125 cases of metastatic pulmonary carcinoma in comparison with more than 100 cases of pulmonary carcinoma revealed that the macroscopic and microscopic distribution of the secondary neoplasms followed closely that of the primary growths.

With these observations in view, the author believes that all tumors presenting alveolar cell arrangement in the lungs are secondary to foci in other organs, or if they are primary in the lungs, that they arise from the basal cells of the bronchi and bronchioles, there being no justification for the assumption that they originate in septal cells.—J. G. K.

Surgical Diseases of the Lung. McGRATH, E. J., and WOODS, F. M. [Coll. of Med., Univ. of Cincinnati, Cincinnati, Ohio] *West Virginia M. J.*, **42**:145-150. 1946.

This is a discussion of surgery of the lung, the indications for such surgery, and the results which can be obtained. Benign tumors of the lung, as simple fibroma or lipoma, are rare. Bronchial adenomas, the nature of which is still a subject of bitter controvery, constitute 5 to 10% of all intrabronchial tumors. Primary carcinoma of the lung constitutes from 8 to 20% of all carcinomas; it is not a rare lesion, as was once believed. Squamous cell carcinoma accounts for 85% of these tumors, adenocarcinoma 10%, and "oat cell" carcinoma 5% of primary pulmonary malignancies. It is important to assume that all unexplained pulmonary lesions in patients past 40 years, however slight, are carcinoma until proved otherwise. X-ray and bronchoscopy, with or without biopsy, are the chief aids in diagnosis; 25 to 30% of cases are, however, not accessible by bronchoscopy, and in these instances exploratory thoracotomy is warranted. The only justifiable therapy is total extirpation of the involved lung, with all the accessible regional lymph nodes; the limits of operability are presented in detail. The authors report a current salvage rate of 54.6%.—V. J. L.

Intrathoracic Hypernephroma Metastases Simulating Primary Pulmonary Disease. Contribution to the Differential Diagnosis in Cases of Hilus Lymphomas. Transpleural Gland Biopsy. RADNER, S. [Lung Clinic, Lund, Sweden] *Acta med. Scandinav.*, **112**:264-276. 1942.

Following a survey of the symptoms of malignant renal tumors, a case of hypernephroma is presented, in which the pulmonary symptoms arising from metastases were the only clinical manifestation, and in which the correct diagnosis was reached at autopsy. X-ray examination revealed pronounced bilateral hilus lymph gland enlargement and parenchymal changes of at first limited, but later gradually increased, diffusion. Two cases are described in which intrathoracic hypernephroma metastases made their first roentgenological appearance in the hilus glands, without simultaneous parenchymal changes. Transpleural gland biopsy is recommended for those cases of hilus lymphoma in which it is urgent to obtain a definite diagnosis for therapy and prognosis.—M. H. P.

Pulmonary Metastasis of Carcinoma Diagnosed by Bronchoscopy. TINNEY, W. S., and MCDONALD, J. R. [Rochester, Minn.] *Minnesota Med.*, **28**:554-558. 1945.

Three cases are presented of metastatic carcinoma that ulcerated through some portion of the tracheobronchial mucosa and were diagnosed by means of bronchioscopic biopsies—R. A. H.

Teratoma of the Anterior Mediastinum in the Group of Military Age. A Study of Sixteen Cases, and a Review of Theories of Genesis. SCHLUMBERGER, H. G. [Army Inst. of Path., Washington, D. C.] *Arch. Path.*, **41**:398-444. 1946.

The clinical course and morphologic characteristics of teratomas have been studied in 16 instances in which the growth occupied the anterior mediastinum. In 10 of these cases the growth was benign and in 6 cancerous. All the patients fell within the military age group of 18 to 38 years; 15 were males; 1 was a female. In the specimens of benign teratoma the most frequently encountered organoid structures were skin, "intestine," "bronchus" and "pancreas." The incidence of well developed pancreas in 6 of the 10 specimens is remarkable in view of its infrequent occurrence in specimens of teratoma of other regions. The specimens of cancerous teratoma of this series were characterized by almost complete absence of ectodermal derivatives, such as skin or nerve tissue. The connective tissue was loose, cellular and may have undergone cancerous change. The cancerous epithelium was arranged as adenocarcinoma in each instance. Well differentiated organoid epithelial structures were absent. Metastases were found in 4 cases.

During the past half century the experimental analysis of morphogenesis has made important advances. Outstanding among these has been the development of the concept of the organizer which holds that substances ("organizers") liberated by one group of cells may determine the differentiation and the organization of other groups of cells. The hypotheses of the genesis of teratomas have been examined in the light of advances in embryology. It is concluded that teratomas of the ovaries and the testes are due to abnormal growth and differentiation of undifferentiated precursors of the germ cells. Extragonadal teratomas, however, are the results of a local dislocation of tissues during embryogenesis. Teratoma of the anterior mediastinum probably arises from tissue dislocations in the anlage of the thymus.—Author's summary.—J. G. K.

Mesothelioma of the Pleura. Report of Case. HERTZOG, A. J. [Minneapolis General Hosp., Minneapolis, Minn.] *Minnesota Med.*, **28**:209-210. 1945.

A case report is given including clinical, laboratory and necropsy findings.—R. A. H.

Primary Cystic Tumor of the Diaphragm. SCOTT, O. B., and MORTON, D. R. [Univ. of Chicago, Chicago, Ill.] *Arch. Path.*, **41**:645-650. 1946.

A case report, with discussion.—J. G. K.

HEART

Hemopericardium from Pericardial Metastatic Carcinoma. RUKSTINAT, G. J. [Loretto Hosp., Chicago, Ill.] *Arch. Path.*, **41**:550-551. 1946.

Report of a case.—J. G. K.

GASTROINTESTINAL TRACT

Carcinoma of the Esophagus. KINSELLA, T. J. [Minneapolis, Minn.] *Minnesota Med.*, **28**:1018-1019. 1945.

A general consideration of the disease with short case reports of 2 patients treated by transthoracic resection and end to end anastomosis of the esophagus and stomach. —R. A. H.

Experiences with the Gastroscope over a Period of Six Years. HOWARD, J. T. [Johns Hopkins Hosp., Baltimore, Md.] *South. M. J.*, **38**:293-302. 1945.

The author summarizes the value of gastroscopy and gives several typical case reports with the findings on gastroscopy. He feels that a negative gastroscopic examination is valueless, and that gastroscopy rarely reveals an ulcer or tumor not demonstrable by x-ray: He reports two patients in the 6-year period in whom a gastric carcinoma was found first by gastroscopic examination and later by x-ray. The gastroscope, used as an adiunct to the x-ray, is of "great diagnostic importance in the study of the coarser lesions, such as ulcers, tumors, and the demonstration of bleeding areas."—W. A. B.

Evaluation of Gastroscopic, Roentgen, Sigmoidoscopic and Laboratory Procedures in 500 Gastrointestinal Cases. MONAT, H. A., and THOMPSON, C. M. [Gastrointestinal Service, U. S. Naval Hosp., St. Albans, L. I., N. Y.] *Rev. Gastroenterol.*, **13**:19-23. 1946.

Patients were subjected to routine psychiatric, gastroscopic, sigmoidoscopic and roentgenologic studies. Roentgen studies were found to be most conclusive in the diagnosis of peptic ulcer or new growth, gastroscopic studies

for the gastritides, and sigmoidoscopic examination for defects of the colon. Evaluation of symptoms, gastric analysis, and the presence of occult blood in the stool often appeared to be unreliable. The series studied includes one case of gastric and one of rectal cancer.—L. J. D.

Carcinoma of the Small Intestines. ROSE, B. T. [Birmingham, England] *Brit. J. Surg.*, **33**:186. 1945.

There was hypertrophy and dilatation below the tumor for which no cause could be found.—E. L. K.

Multiple Malignant Argentaffin (or Carcinoid) Tumors of the Small Bowel with Disseminated Metastasis. WATZ, C. E. [St. Paul, Minn.] *Minnesota Med.*, **28**:558-559. 1945.

The literature is reviewed and a case is presented.—R. A. H.

Carcinoma of the Ileum. NELSON, H. [Minneapolis, Minn.] *Minnesota Med.*, **28**:396-398. 1945.

A review is given of the pathology and clinical picture with the report of 2 cases.—R. A. H.

The Radical Operation for Carcinoma of the Rectosigmoid. WILENSKY, A. O. [New York, N. Y.] *Med. Rec.*, **159**:95-98. 1946.

A perineal approach with preservation of the sphincter can be achieved only with carcinoma above the line of the anal canal because of the lymphatic drainage channels; this procedure is feasible only with well-localized malignancy and in the absence of widespread lymph node or distant metastasis. The difficulties of primary healing of intestinal anastomosis and infection have been overcome by greater operative skill and chemotherapy.

The 6 commonest methods of operation for dealing with carcinoma of the rectosigmoid are summarized; regardless of the technic employed the success of the operation depends upon adequate mobilization, a long mobile sigmoid, absence of sigmoiditis and mesosigmoiditis, and an adequate blood supply.—V. J. L.

LIVER

Multiple Malignant Hemangiomas of the Liver. ANDRIES, G. H., and KAUMP, D. H. [Providence Hosp., Detroit, Mich.] *Am. J. Clin. Path.*, **14**:489-494. 1944.

A case report.—J. G. K.

Carcinoma of the Ampulla of Vater. MAXEINER, S. R. [Minneapolis, Minn.] *Minnesota Med.*, **28**:225-227. 1945.

A review is given of surgical treatment with the detailed report of one case.—R. A. H.

Primary Carcinoma of the Liver of a Dog. BOOKER, W. M., and WEBB, A. C. [Howard Univ. Sch. of Med., Washington, D. C.] *Arch. Path.*, **41**:548-549. 1946.

Report of a case.—J. G. K.

SPLEEN

Splenic Hemangiosarcoma. A Case with Lymphatic and Vascular Metastases. BAUER, D. deF., and STANFORD, W. R. [Duke Univ. Sch. of Med., Duke Hosp., and Watts Hosp., Durham, N. C.] *Arch. Path.*, **41**:668-673. 1946.

Report of a case with discussion of several additional cases from the literature is given.—J. G. K.

BONE AND BONE MARROW

Multiple Myeloma. Report of Three Cases. HERTZOG, A. J. [Minneapolis General Hosp., Minneapolis, Minn.] *Minnesota Med.*, **27**:1011-1013. 1944.

A report is made of three cases, giving salient clinical, laboratory and sternal biopsy findings.—R. A. H.

Round Cell Tumor of Bone Resembling Ewing's Tumor. REEVES, R. J. [Duke Univ. Sch. of Med., Durham, N. C.] *South. M. J.*, **38**:302-306. 1945.

Reports of 2 cases occurring in girls, 6 and 9 years old is presented. One child, who had lung metastases with a primary lesion in the sixth rib, showed dramatic improvement with roentgen therapy. There was no recurrence when she was last seen 11 years later. The other patient had a primary tumor in the region of the left lower premolars. After local surgical excision she also received roentgen therapy, but there were repeated recurrences in the mandible after 2 years, and extension to the cervical nodes. After the second recurrence, removal of the left mandible and a radical neck dissection were performed. Tumors appeared in the lungs 8 years after the first excision, and regressed completely following x-ray therapy. She has now remained free from metastases for 2 years.—W. A. B.

Surgery of the Mandible: The Ameloblastoma. BYARS, L. T., and SARNAT, B. G. [Washington Univ. Med. Sch., St. Louis, Mo.] *Surg., Gynec. & Obst.*, **81**:575-584. 1945.

Surgical procedures for various ameloblastomas are described in detail.—J. G. K.

Ostéosarcome et Maladie de Paget. [Osteosarcoma and Paget's Disease.] LAYANI, F., and OLIVIER, C. [Paris, France] *Presse Méd.*, **54**:145-146. 1946.

A case report. Osteosarcoma of the femur in a 80-year-old man suffering from osteitis deformans.—C. A.

MUSCLE AND TENDON

Myoblastoma (Granular Cell Myoblastoma or Myoblastic Myoma). CRANE, A. R., and TREMBLAY, R. G. [Norfolk General Hosp., Norfolk, Va., and St. John's Hosp., Brooklyn, N. Y.] *Am. J. Path.*, **21**:357-372. 1945.

Five new cases are reported, along with a discussion of 157 cases from the literature.—J. G. K.